AKADEMIJA MEDICINSKIH ZNANOSTI HRVATSKE
KOLEGIJ JAVNOG ZDRAVSTVA, ODBOR ZA PRAĆENJE REZISTENCIJE BAKTERIJA NA ANTIBIOTIKE U REPUBLICI HRVATSKOJ
CROATIAN ACADEMY OF MEDICAL SCIENCES
PUBLIC HEALTH COLLEGIUM, COMMITTEE FOR ANTIBIOTIC RESISTANCE SURVEILLANCE IN CROATIA

KLINIKA ZA INFEKTIVNE BOLESTI "DR. F. MIHALJEVIĆ"
REFERENTNI CENTAR ZA PRAĆENJE REZISTENCIJE BAKTERIJA NA ANTIBIOTIKE MINISTARSTVA ZDRAVLJA
UNIVERSITY HOSPITAL FOR INFECTIOUS DISEASES "DR. F. MIHALJEVIĆ"
REFERENCE CENTER FOR ANTIBIOTIC RESISTANCE SURVEILLANCE, CROATIAN MINISTRY OF HEALTH

Osjetljivost i rezistencija bakterija na antibiotike u Republici Hrvatskoj u 2013.g.

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SADRŽAJ

PREDGOVOR:

Problem rezistencije bakterija na antibiotike je obilježio medicinu kraja 20. i početka 21. stoljeća. Strah da će infekcije uzrokovane bakterijama otpornim na sve antibiotike ugroziti liječenje infektivnih bolesti te primjenu invazivnih metoda dijagnostike i liječenja mnogih bolesti motivirao je ne samo stručna društva već i vlade mnogih zemalja na brojne akcije kontrole širenja rezistencije. Praćenje potrošnje antibiotika i rezistencije bakterija na antibiotike u lokalnim sredinama te na nacionalnoj razini postalo je osnova programa kontrole širenja rezistencije u Europi. U Hrvatskoj je sistematsko praćenje rezistencije na nacionalnoj razini započelo 1996.g. osnutkom Odbora za praćenje rezistencije bakterija na antibiotike u RH pri Kolegiju za javno zdravstvo Akademije medicinskih znanosti Hrvatske (AMZH). Odbor danas okuplja voditelje više od 90% mikrobioloških laboratorija te stručnjake iz područja infektologije i kliničke farmakologije koji se posebno bave antimikrobnom terapijom. Ovako organizirana mreža mikrobioloških laboratorija spremno se uključila u europski projekt the European Antimicrobial Resistance Surveillance System (EARSS), koji je nakon deset godina rada, 2009.g. prerastao u kontinuirani program EARS-Net Europskog centra za prevenciju i kontrolu bolesti (engl. "European Center for Disease Control", ECDC). Uzročno posljedična veza između potrošnje antibiotika i rezistencije bakterija je logična i često dokazivana. Više od 90% antibiotika se potroši izvanbolnički, vrlo često na blage infekcije ili infekcije koje nisu uzrokovane bakterijama. U bolnicama nesvrsishodna uporaba antibiotika je potencirana nedostatkom timova za rukovođenje antimikrobnom terapijom (engl. "antibiotic stewardship teams"). Internacionalno usporedivo praćenje potrošnje antibiotika u Hrvatskoj započelo je 2002.g. uključivanjem u the European Surveillance of Antimicrobial Consumption (ESAC) projekt, koji je 2010.g. prerastao u kontinuirani program ESAC-Net ECDC-a. U okviru Odbora osnovana je 2003.g. hrvatska podružnica internacionalne organizacije The Alliance for the Prudent Use of Antibiotics (APUA), a 2011.g. Povjerenstvo za metodologiju određivanja osjetljivosti na antibiotike koje je formalno preuzelo zadatak redovitog prilagođavanja European Committee for Antimicrobial Sensitivity Testing (EUCAST) preporuka i standarada rutinskoj dijagnostici u Hrvatskoj. Standardizacija testiranja osjetljivosti na antibiotike u mreži hrvatskih laboratorija je od samog početka bio jedan od prioriteta rada Odbora. U tu svrhu uvedene su i redovite vanjske kontrole testiranja osjetljivosti koje pokazuju visoki stupanj harmonizacije i prihvaćanja novosti u izradi i interpretaciji testova osjetljivosti među hrvatskim laboratorijima. Velika podrška radu Odbora je bliska suradnja s Referentnim centrom (RC) Ministarstva zdravlja (MZ) za praćenje rezistencije bakterija na antibiotike, koji je osnovan 2003.g. pri Klinici za infektivne bolesti "Dr. F. Mihaljević". Sve aktivnosti iz područja kontrole širenja rezistencije u Hrvatskoj koordinira Interdisciplinarna sekcija za kontrolu rezistencije na antibiotike (ISKRA), Ministarstva zdravlja, koja je osnovana 2006.g. U okviru rada ISKRA-e podaci o potrošnji antibiotika i rezistenciji bakterija u Hrvatskoj se koriste za druge aktivnosti usmjerene na racionalizaciju uporabe antibiotika, kao što su edukacija, razvoj smjernica i javne kampanje. Podaci o rezistenciji i potrošnji antibiotika iz ove redovite publikacije Odbora i Referentnog centra za praćenje rezistencije prezentiraju se na mnogim stručnim i znanstvenim skupovima, od kojih je u 2013.g. najznačajniji bio CROCMID, zajednički kongres hrvatskih mikrobiologa i infektologa.

Arjana Tambić Andrašević
Predsjednica Odbora za praćenje rezistencije bakterija na antibiotike u RH

PREFACE:

The problem of antibiotic resistance has significantly marked end of the 20th and beginning of the 21st century. Fear that bacteria resistant to all antibiotics will jeopardize the treatment of infectious diseases and outcomes of diagnostic and invasive treatment procedures for many other diseases has motivated not only professional societies but also governments of many countries to start various actions towards controlling the spread of resistance. Surveillance of antimicrobial consumption and antimicrobial resistance at local and national level became the cornerstone of antimicrobial resistance control program in Europe. In Croatia continuous antibiotic resistance surveillance at national level started in 1996 when the Croatian Committee for Antibiotic Resistance Surveillance (CARS) was founded at the Public Health Collegium of the Croatian Academy of Medical Sciences (CAMS). Today the Committee gathers heads of more than 90% of microbiology laboratories in the country and infectious diseases and clinical pharmacology experts in the field of antimicrobial therapy. This network of microbiology laboratories readily joined the European Antimicrobial Resistance Surveillance System (EARSS) project which was transferred in 2009 into a continuous EARS-Net program of the European Centre for Disease Control (ECDC). Correlation between antibiotic consumption and development of resistance is logical and has been frequently documented. More than 90% of antibiotics are prescribed ambulatory, often for mild or non-bacterial infections. In hospitals misuse of antibiotics is largely due to the lack of antibiotic stewardship teams. Internationally comparable surveillance of antibiotic consumption in Croatia started in 2002 by joining the European Surveillance of Antimicrobial Consumption (ESAC) project which evolved into a continuous ESAC-Net ECDC program in 2010. The Committee founded the Croatian Chapter of the Alliance for the Prudent Use of Antibiotics (APUA) in 2003 and a Subcommittee for antibiotic sensitivity testing (AST) methodology in 2011. The AST Subcommittee is officially in charge of adapting and regularly updating the European Committee for Antimicrobial Sensitivity Testing (EUCAST) recommendations and standards. AST standardization was one of the priorities of the CARS from the very beginning of microbiology network building and has led to the introduction of the regular external quality control which repeatedly demonstrates high level of compliance with AST methodology and interpretation updates. A big support to the CARS activities is provided by the Ministry of Health Reference Centre (RC) for Antibiotic Resistance Surveillance which was established in 2003 at the University Hospital for Infectious Diseases "Dr. F. Mihaljević". All the activities related to antibiotic resistance control are coordinated by the Croatian intersectorial coordination mechanism (ICM) at the Ministry of Health, the so called „Interdisciplinarna sekcija za kontrolu rezistencije na antibiotike" (ISKRA) which was founded in 2006. Owing to the ISKRA coordination antibiotic resistance and antibiotic consumption data are incorporated into other activities targeting antibiotic resistance control, such as education, guidelines development and public campaigns. Antibiotic resistance and consumption data reported in this regular joint publication of CARS and RC are frequently presented at many professional and scientific meetings of which the most remarkable one in 2013 was CROCMID, the joint congress of Croatian clinical microbiologists and infectious diseases doctors.

Arjana Tambić Andrašević
President of the Committee for Antibiotic Resistance Surveillance in Croatia

POGLAVLJE/CHAPTER 1.

REZISTENCIJA BAKTERIJSKIH IZOLATA U 2013. GODINI
ANTIBIOTIC RESISTANCE IN 2013

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UVOD:

U skladu s postavkama Nacionalnog programa kontrole širenja otpornosti bakterija na antibiotike, 2009 – 2014 Odbor za praćenje rezistencije bakterija na antibiotike Akademije medicinskih znanosti Hrvatske (AMZH) i Referentni centar za praćenje rezistencije bakterija na antibiotike Ministarstva zdravlja (MZ) pri Klinici za infektivne bolesti "Dr. Fran Mihaljević" zaduženi su za provođenje praćenja rezistencije u Hrvatskoj. Svrha prikupljanja i objavljivanja ovih podataka je racionalno primjenjivanje empirijske antimikrobne terapije te širenje svijesti o stupnju rezistencije i količini potrošnje antibiotika u pojedinim sredinama u Hrvatskoj. Dobro organizirana mreža mikrobioloških laboratorija, pravodobno uočavanje izolata s novim mehanizmima rezistencije i slanje izolata rijetkog i neuobičajenog fenotipa u referentni centar na retestiranje i daljnju karakterizaciju pokazalo se izuzetno bitnim u kontroliranju početnog širenja multiplorezistentnih uzročnika poput enterobakterija otpornih na karbapeneme. Podaci o izolatima rijetkog fenotipa, prikupljeni u referentnom centru, opisani su u zasebnom poglavlju. U zasebnom poglavlju prikazani su i invazivni izolati određenih bakterijskih vrsta za koje se prikupljaju i osnovni demografski podaci.

INTRODUCTION:

According to the National strategy for antibiotic resistance control, 2009 – 2014 the Croatian Committee for Antibiotic Resistance Surveillance of the Croatian Academy of Medical Sciences (CAMS) and the Reference Centre for Antibiotic Resistance Surveillance of the Croatian Ministry for Health (MH) at the University Hospital for Infectious Diseases "Dr Fran Mihaljević" are given the responsibility to conduct continuous surveillance of antibiotic resistance in Croatia. This surveillance data should enable rational use of antibiotics in empirical therapy and will help to raise awareness about resistance and antibiotic consumption rates in different regions of Croatia. A well organized microbiology laboratory network, timely recognition of isolates with novel resistance mechanisms and sending such isolates to reference centre for retesting and further characterization proved to be essential in controlling the initial spread of multiply resistant organisms such as carbapenem resistant enterobacteriaceae. Isolates with rare phenotypes, collected at the reference centre, are described in a separate chapter of this publication. Invasive isolates, for which basic demographic data are collected, are also described separately.

MATERIJALI I METODE:

Globalno praćenje rezistencije

U praćenje su uključeni svi izolati dogovorenih bakterijskih vrsta izolirani iz kliničkih materijala u razdoblju od 1.10. do 31.12.2013.g. Rezultati za izolate streptokoka grupe A, salmonela, šigela, kampilobaktera i anaerobnih bakterija prikupljaju se, zbog malog broja izolata, tijekom cijele godine, od 1.1. do 31.12.2013. Podatke za 2013.g. podnjelo je 40 centara (popis u legendi za tablice), što obuhvaća >90% populacije u Hrvatskoj.

Osnovna načela metodologije praćenja rezistencije, kojih se pridržavaju svi koji u praćenju sudjeluju, uključuju:

a. u ispitivanom razdoblju svi izolati određene bakterijske vrste testiraju se na sve antibiotike predviđene za tu vrstu. Od 2010.g. na snazi je dogovor da iznimka za ovo pravilo bude testiranje osjetljivosti *P. aeruginosa* i *A. baumannii* na kolistin. Zbog skupoće testiranja preporuča se da se kolistin testira samo kod izolata neosjetljivih na karbapeneme.

b. antibiotici predviđeni za određenu vrstu navedeni su u formularima za praćenje rezistencije za tekuću godinu

c. u ispitivanom razdoblju s dogovorenom paletom antibiotika testiraju se svi izolati iz kliničkih materijala ili barem prvih 100 uzastopnih izolata

d. iz podataka se isključuju duplikatni sojevi, definirani kao izolati iste bakterijske vrste, izolirani u istog pacijenta, u bilo kojem uzorku, u razdoblju od 30 dana.

Laboratoriji svoje podatke šalju na obradu u Referentni centar za praćenje rezistencije, Klinika za infektivne bolesti "Dr. F. Mihaljević". Na svakom formularu su označeni neuobičajeni fenotipovi na koje treba obratiti pažnju i poslati na retestiranje u Referentni centar. Takvi izolati od posebnog interesa uključuju:

1. pneumokoke rezistentne na norfloksacin
2. stafilokoke rezistentne na vankomicin i / ili linezolid
3. enterokoke rezistentne na vankomicin
4. *H.influenzae* rezistentan na ko-amoksiklav i / ili cefalosporine III generacije (engl. "beta-lactamase negative ampicillin resistant", BLNAR sojeve)
5. izolate *E. coli* i *K. pneumoniae* koji ne proizvode beta-laktamaze proširenog spektra (engl. "extended spectrum beta-lactamases", ESBL), a rezistentni su na jedan od cefalosporina III ili IV generacije
6. enterobakterije rezistentne na bilo koji od karbapenema

Tijekom 2013.g. korišteni su za testiranje i interpretaciju nalaza standardi europskog odbora, European Committee for Antimicrobial Sensitivity Testing (EUCAST) standardi (verzija 3.0). U testiranju većina laboratorija koristi disk difuzijsku metodu, a određivanje minimalnih inhibitornih koncentracija (MIK) se koristi za određivanje osjetljivosti na penicilin kod pneumokoka smanjene osjetljivosti na penicilin, za određivanje osjetljivosti stafilokoka na glikopeptide te pseudomonasa i acinetobaktera na kolistin.

Preporuka Odbora je da se izolati *A. baumanii* i *P. aeruginosa* rezistentni na jedan, ali ne i oba karbapenema retestiraju određujući MIK za imipenem i meropenem. Minimalne inhibitorne koncentracije su određivane E-test metodom.

Osjetljivost anaerobnih bakterija testirana je određivanjem MIK-a koristeći E-test metodu ili mikrodiluciju u bujonu.

Vrste bakterija i ispitani antibiotici navedeni su u tablicama u daljnjem tekstu.

Ciljane studije

Podaci o osjetljivosti *M. tuberculosis* su obrađivani u nacionalnom laboratoriju za tuberkulozu, Hrvatskog zavoda za javno zdravstvo. Rezistencija *M. tuberculosis* je opisana u posebnom poglavlju ove publikacije.

Od početka suradnje s European Antimicrobial Resistance Surveillance System (EARSS) projektom 2001.g. Odbor je počeo posebno obrađivati rezistenciju u invazivnih izolata (iz krvi i likvora) bakterijskih vrsta *S. pneumoniae*, *S. aureus, E. faecalis, E. faecium, E. coli, K. pneumoniae, P. aeruginosa*, a od 2013.g. i *Acinetobacter baumannii*. Za ove izolate RC za praćenje rezistencije prikuplja i obrađuje demografske podatke pacijenata, a u svrhu detaljnije analize invazivni izolati enterokoka, stafilokoka i *P. aeruginosa* šalju se u Zavod za kliničku i molekularnu mikrobiologiju Kliničkog bolničkog centra Zagreb, a invazivni izolati pneumokoka, *E. coli, K. pneumoniae* i *Acinetobacter baumannii* u Zavod za kliničku mikrobiologiju Klinike za infektivne bolesti "Dr. F. Mihaljević". Otkad je 2010.g. EARSS prešao u EARS-Net, mrežu koja čini jedan segment The European Surveillance System (Tessy) Europskog centra za kontrolu bolesti (engl. "European Center for Disease Control", ECDC), RC za praćenje rezistencije je dužan slati podatke o invazivnim izolatima u Tessy sustav. Podaci o invazivnim izolatima od početka praćenja do 2013.g. prikazani su u zasebnom poglavlju ove publikacije.

Hrvatska se 2001.g. uključila u europski projekt European Surveillance of Antimicrobial Consumption (ESAC) i tako počela pratiti potrošnju antibiotika izraženu u definiranim dnevnim dozama na 1000 stanovnika dnevno (DDD/TID). Nakon prelaska ESAC projekta u ESAC-Net program ECDC-a 2011.g. Hrvatska je dužna slati podatke o bolničkoj i izvanbolničkoj potrošnji antimikrobnih lijekova u Tessy sustav ECDC-a. Podaci o potrošnji antibiotika u Hrvatskoj u 2013.g. su objavljeni kao posebno poglavlje ove publikacije, a uključuju i detaljniju analizu bolničke potrošnje antibiotika koja se detaljnije počela pratiti od 2006.g. u sklopu APUA Croatia inicijative i u skladu s naputcima ISKRA-e.

U posebnom poglavlju prikazan je osvrt na sojeve poslane na retestiranje u Referentni centar za praćenje rezistencije. Iz ovog poglavlja bolje se može uočiti problem multiplorezistentnih bakterija u Hrvatskoj s obzirom da se rijetki izolati s novim mehanizmima rezistencije često ne prikazuju kao postotak u velikom broju izolata obrađenih u masovnom praćenju.

MATERIALS AND METHODS:

Global surveillance

Global antibiotic resistance surveillance includes all clinical isolates of designated bacterial species isolated from 1 October till 31 December, 2013. Exceptionally, data for group A streptococci, salmonellae, shigellae, campylobacters and anaerobic bacteria are collected throughout the year due to the small number of isolates. In 2013 a total of 40 centres took part in antibiotic resistance surveillance (names of the centres are listed in the legend to the tables) which makes a catchment population of >90%.

Basic principles of resistance surveillance methodology, obligatory for all the participants, include the following:

a. during the study period all isolates of a given species are to be tested against all the designated antibiotics. Since 2010 the exception from this rule is applied for *P. aeruginosa* and colistin. Because of the high cost for colistin testing it was decided that colistin should be tested only in pseudomonas and acinetobacter isolates that are nonsusceptible to carbapenems.

b. antibiotics designated to a particular bacterial species are listed on the antibiotic resistance surveillance form for the current year

c. during the study period a designated set of antibiotics is to be tested against all or at least first 100 consecutive clinical isolates of each species

d. copy isolates are defined as isolates of the same species collected from the same patient within a 30 day period and they are excluded from the data

Laboratories send their data for analysis to the Croatian Reference Centre for Antibiotic Resistance Surveillance, University Hospital for Infectious Diseases "Dr. F. Mihaljević". Unusual and alert phenotypes are indicated on every collection form and they are to be referred to the Reference centre. The alert microorganisms include the following:

1. pneumococci resistant to norfloxacin
2. staphylococci resistant to vancomycin or linezolid
3. vancomycin resistant enterococci
4. *H.influenzae* resistant to co-amoxiclav and / or III generation cephalosporins (beta-lactamase negative ampicillin resistant, BLNAR strains)
5. *E.coli* and *K.pneumoniae* isolates that do not produce extended spectrum beta-lactamases (ESBL) but are resistant to one of the III or IV generation cephalosporins
6. carbapenem resistant enterobacteriaceae

In 2013 EUCAST standards (version 3.0) were used as official methodology for sensitivity testing. Disk diffusion method is the most widely used sensitivity testing method in Croatia and minimal inhibitory concentration (MIC) testing is used for detection of penicillin resistance in penicillin non-susceptible pneumococci, glycopeptide resistance in staphylococci and colistin resistance in pseudomonas and acinetobacter.

The Committee recommendation is that for *A. baumannii* and *P. aeruginosa* isolates resistant to one but not to both carbapenems MICs of imipenem and meropenem should be determined. MIC testing was done by E-test.

Antibiotic sensitivity in anaerobic bacteria was determined by E-test or broth dilution method.

Bacterial species and antibiotics tested are listed in tables in further text.

Focused studies

Data on *M. tuberculosis* were processed in the National Laboratory for Tuberculosis at the Croatian Public Health Institute. Resistance in *Mycobacterium tuberculosis* is described in a separate chapter of this publication.

Ever since Croatia joined the European Antimicrobial Resistance Surveillance System (EARSS) project in 2001 the Committee started to collect data on invasive isolates (isolates from blood and cerebrospinal fluid) of *S. pneumoniae, S. aureus, E. faecalis, E. faecium, E. coli, K. pneumoniae, P. aeruginosa* and since 2013 *Acinetobacter baumannii*. For these isolates Reference centre (RC) for resistance surveillance collects and analyses patient demographic data and for the purpose of more detailed analysis invasive isolates of enterococci, staphylococci and *P.aeruginosa* are regularly sent to the Institute for Clinical and Molecular Microbiology, Clinical Hospital Centre Zagreb and invasive pneumococci, *E. coli*, *K. pneumoniae* and *A.baumannii* are sent to the Department of Clinical Microbiology, University Hospital for Infectious Diseases "Dr. F. Mihaljević". Since EARSS was transferred to EARS-Net, a part of The European Surveillance System (Tessy), a global European Centre for Disease Control (ECDC) surveillance network, RC for resistance surveillance is obliged to send Croatian resistance data to Tessy. Data on invasive isolates from the beginning of surveillance until 2013 are presented in a separate chapter of this publication.

Croatia joined the European Surveillance of Antimicrobial Consumption (ESAC) project in 2001 and started to analyze antibiotic consumption data expressed as defined daily doses per thousand inhabitants daily (DDD/TID). After ESAC transition to the ECDC ESAC-Net in 2011 Croatia is obliged to send hospital and ambulatory antibiotic consumption data to ECDC Tessy. Antibiotic consumption data in 2013 are presented in a separate chapter of this publication and they also include a more detailed analysis of antibiotic consumption in hospitals which was initiated by the APUA Croatia Chapter in 2006 and is in line with ISKRA requirements.

A special chapter deals with the isolates sent for retesting to the Reference Centre for Antibiotic Resistance Surveillance. This detailed report provides a better insight in the spread of multiply resistant bacteria in Croatia as the presence of some strains with novel resistance mechanisms is still not seen as increase in resistance rates.

REZULTATI

U praćenju rezistencije u 2013.g. sudjelovalo je 40 centara u Hrvatskoj. Prosječni rezultati za Hrvatsku i rezultati za pojedinačne centre prikazani su u tablicama i grafovima u daljnjem tekstu. Rezultati laboratorija koji su prijavili manje od 30 izolata pojedine bakterijske vrste smatraju se nepouzdanim podacima za taj centar, ali su uvršteni u tablice i uključeni su u zbirne rezultate za RH. Podaci o izolatima malo vjerojatnog fenotipa koji nisu potvrđeni u jednom od centralnih laboratorija označeni su zvjezdicom kao nepotvrđeni i ne smatraju se važećima.

Zbog malog broja izolata u ispitivanom razdoblju neki centri su ispitivanje proširili na cijelu godinu, a neki su zbog različitih razloga odstupali od predviđenog razdoblja praćenja. Odstupanja od predviđenog razdoblja praćenja uključuju:

- KA OB, PK OŽB i KT MAGD su za sve vrste prikazali rezultate za cijelu godinu
- GS ZZJZ je za *K. pneumoniae* prikazao rezultate za cijelu godinu
- KA ZZJZ je za *E. faecium*, *P. aeruginosa* i *Enterobacter, Serratia* i *Citrobacter* prikazao rezultate za cijelu godinu
- PŽ ZZJZ je za *S. pneumoniae, E. faecalis* i *P. mirabilis* prikazao rezultate za cijelu godinu
- ČK ZZJZ je za *E. faecium* prikazao rezultate za cijelu godinu
- PU ZZJZ je za *H.influenzae* i *A. baumannii* prikazao rezultate za cijelu godinu
- ŠI ZZJZ je za *A. baumannii* prikazao rezultate za cijelu godinu
- VK ZZJZ je za *S. pneumoniae*, *S. aureus*/MSSA, *S. aureus*/MRSA, *H.influenzae* i *A. baumannii* prikazao rezultate za cijelu godinu
- ZG KBM je za *S. pneumoniae* i *H.influenzae* prikazala rezultate za cijelu godinu
- ZG KIB je za *H.influenzae* prikazala rezultate za cijelu godinu

Pet laboratorija je prijavilo izolaciju šigela: DU ZZJZ *Sh. flexneri* (1); KC ZZJZ *Sh.sonnei* (1); OG OB *Sh.sonnei* (1); OS ZZJZ *Sh. flexneri* (1); RI NZZJZ *Sh.sonnei* (22), *Sh.flexneri* (1). Ukupno je tijekom 2013.g. izolirano 27 šigela.

U 2013.g. ukupno je obrađeno 668 anaerobnih bakterija, 295 gram-pozitivnih i 373 gram-negativnih anaeroba iz 20 centara : ČK ZZJZ gram-pozitivni anaerobi (9), gram-negativni anaerobi (33); KA OB gram-pozitivni anaerobi (19), gram-negativni anaerobi (14); KC ZZJZ gram-negativni anaerobi (1); KT KZKB gram-pozitivni anaerobi (2), gram-negativni anaerobi (1); OS ZZJZ gram-pozitivni anaerobi (7), gram-negativni anaerobi (10); PU ZZJZ gram-negativni anaerobi (3); RI KBC gram-pozitivni anaerobi (43), gram-negativni anaerobi (21); SB ZZJZ gram-pozitivni anaerobi (8), gram-negativni anaerobi (11); SK ZZJZ gram-pozitivni anaerobi (2), gram-negativni anaerobi (7); ST KBC gram-pozitivni anaerobi (29), gram-negativni anaerobi (47); ŠI ZZJZ gram-pozitivni anaerobi (28), gram-negativni anaerobi (33); VK ZZJZ gram-pozitivni anaerobi (2), gram-negativni anaerobi (3); VT ZZJZ gram-pozitivni anaerobi (1), gram-negativni anaerobi (19); VŽ ZZJZ gram-pozitivni anaerobi (40), gram-negativni anaerobi (45); ZD ZZJZ gram-pozitivni anaerobi (9), gram-negativni anaerobi (20); ZG KBC gram-pozitivni anaerobi (12), gram-

negativni anaerobi (38); ZG KBM gram-pozitivni anaerobi (16), gram-negativni anaerobi (17); ZG KIB gram-pozitivni anaerobi (14), gram-negativni anaerobi (24); ZG KDB gram-pozitivni anaerobi (53), gram-negativni anaerobi (25); ZG KBSD gram-pozitivni anaerobi (1), gram-negativni anaerobi (1).

RESULTS

Forty centres took part in antibiotic resistance surveillance in Croatia in 2013. Average data for Croatia and results for individual laboratories are presented in tables and figures further in the text. Results of the laboratories that reported less than 30 isolates of a single bacterial species were included in tables as to add to the total number for Croatia, but were flagged as not reliable resistance rate data for that individual centre. Where isolates of less probable phenotype were reported without being sent to a central laboratory for retesting, data were flagged as not retested centrally and these data are not considered to be reliable.

Due to low numbers of isolates in the surveillance period some centres expanded surveillance to the whole year and some centres reported different surveillance periods for various reasons. Deviations from official surveillance periods were reported as follows:

- KA OB, PK OŽB and KT MAGD reported data for the whole year for all species
- GS ZZJZ reported data for *K. pneumoniae* for the whole year
- KA ZZJZ reported data for *E. faecium*, *P. aeruginosa* and *Enterobacter, Serratia, Citrobacter* group for the whole year
- PŽ ZZJZ reported data for *S. pneumoniae, E. faecalis* and *P. mirabilis* for the whole year
- ČK ZZJZ reported data for *E. faecium* for the whole year
- PU ZZJZ reported data for *H.influenzae* and *A. baumannii* for the whole year
- ŠI ZZJZ reported data for *A. baumannii* for the whole year u
- VK ZZJZ reported data for *S. pneumoniae, S. aureus/*MSSA, *S. aureus*/MRSA, *H.influenzae* and *A. baumannii* for the whole year
- ZG KBM reported data for *S. pneumoniae* and *H.influenzae* for the whole year
- ZG KIB reported data for *H.influenzae* for the whole year

Five laboratories reported shigella isolates: DU ZZJZ *Sh. flexneri* (1); KC ZZJZ *Sh.sonnei* (1); OG OB *Sh.sonnei* (1); OS ZZJZ *Sh. flexneri* (1); RI NZZJZ *Sh.sonnei* (22), *Sh.flexneri* (1). Altogether 27 shigella isolates were reported in 2013.

In 2013 altogether 668 anaerobic bacteria were isolated, 295 gram-positives and 373 gram-negatives. They were isolated in 20 centres : ČK ZZJZ gram-positive anaerobes (9), gram-negative anaerobes (33); KA OB gram-positive anaerobes (19), gram-negative anaerobes (14); KC ZZJZ gram-negative anaerobes (1); KT KZKB gram-positive anaerobes (2), gram-negative anaerobes (1); OS ZZJZ gram-positive anaerobes (7), gram-negative anaerobes (10); PU ZZJZ gram-negative anaerobes (3); RI KBC gram-positive anaerobes (43), gram-negative anaerobes (21); SB ZZJZ gram-positive anaerobes (8), gram-negative anaerobes (11); SK ZZJZ gram-positive anaerobes (2), gram-negative anaerobes (7); ST KBC gram-positive anaerobes (29), gram-negative anaerobes (47); ŠI ZZJZ gram-positive anaerobes (28), gram-negative anaerobes (33); VK ZZJZ gram-positive anaerobes (2), gram-negative anaerobes (3); VT ZZJZ gram-positive anaerobes (1), gram-negative anaerobes (19);

VŽ ZZJZ gram-positive anaerobes (40), gram-negative anaerobes (45); ZD ZZJZ gram-positive anaerobes (9), gram-negative anaerobes (20); ZG KBC gram-positive anaerobes (12), gram-negative anaerobes (38); ZG KBM gram-positive anaerobes (16), gram-negative anaerobes (17); ZG KIB gram-positive anaerobes (14), gram-negative anaerobes (24); ZG KDB gram-positive anaerobes (53), gram-negative anaerobes (25); ZG KBSD gram-positive anaerobes (1), gram-negative anaerobes (1).

DISKUSIJA

Penicilin je zbog svoje visoke djelotvornosti i uskog spektra lijek izbora za liječenje streptokoknih infekcija od kojih je najčešća streptokokna grlobolja. Makrolidi su alternativa penicilinu u osoba preosjetljivih na penicilin. I dok rezistencija streptokoka grupe A na penicilin još uvijek nije opisana, rezistencija na makrolide iznosi 10% što je još uvjek u granicama vrijednosti prethodnih godina (13% u 2008. godini, 9% u 2009. godini, 8% u 2010. godini, 7% u 2011. godini, 9% u 2012. godini). Slično kao i prošle godine rezistencija na klindamicin je bila konstitutivna u 4% izolata, a inducibilna u 1% izolata. Dogovorno u Hrvatskoj se inducibilna rezistencija streptokoka na klindamicin u nalazu izdaje kao osjetljivost uz opasku da se tijekom dulje terapije može razviti rezistencija na klindamicin.

Iako se računa da je otprilike 10% infekcija gornjih dišnih puteva bakterijske etiologije oko 75% antibiotika se potroši upravo za liječenje respiratornih infekcija. Činjenica da bakterije koje koloniziraju sluznicu gornjih dišnih puteva ujedno predstavljaju i najčešće respiratorne patogene često zavodi kliničare i potiče ih na prekomjernu uporabu antibiotika. Najveći broj izolata pneumokoka i hemofilusa prikazanih u ovom poglavlju potječe iz briseva i aspirata nazofarinksa te je upitnog kliničkog značenja no zbog velikog broja izolata ovi podaci, ipak, pružaju dobar uvid u kretanje rezistencije među bakterijama koje koloniziraju gornji dišni sustav. Rezistencija na antibiotike u invazivnih, klinički neupitno značajnih izolata pneumokoka je obrađena u drugom poglavlju ove publikacije. U većini međunarodnih smjernica amoksicilin predstavlja prvi lijek izbora u liječenju bakterijske upale srednjeg uha zbog dobre učinkovitosti na pneumokoke i *Haemophilus influenzae*. Rezistencija *H. influenzae* na amoksicilin u 2013.g. iznosi 17% što je dosta više negoli prethodnih godina (9% u 2006.g., 11% u 2007.g., 8% u 2008.g., 10% u 2009.g., 11% u 2010.g., 13% u 2011.g. i 2012.g.). Porast rezistencije nakon 2010.g. može se djelomično pripisati uvođenju osjetljivijeg EUCAST standarda za amoksicilin u 2011.g.

Otpornost pneumokoka na beta-laktamske antibiotike se može uobičajenom disk difuzijskom metodom odrediti samo do razine prepoznavanja neosjetljivosti na penicilin. Za daljnje razlučivanje visoke i umjerene rezistencije na penicilin te rezistencije na druge beta-laktamske antibiotike potrebno je odrediti minmalne inhibitorne koncentracije (MIK) za pojedinačne antibiotike što se u rutini radi samo za invazivne izolate. Neosjetljivost na penicilin podrazumijeva da takvi izolati ne podliježu terapiji penicilinom samo ako se radi o infekcijama središnjeg živčanog sustava (SŽS) no kliničari često neosjetljivost poistovječuju s rezistencijom što ih čini nesklonima koristiti penicilin i kod infekcija koje ne uključuju SŽS. Granične koncentracije za beta-laktame su se s vremenom mijenjale i u američkim (Clinical and Laboratory Standards Institute, CLSI) i u europskim (EUCAST) standardima s težnjom da se što preciznije razluči razina rezistencije koja je klinički značajna pri različitim kliničkim indikacijama. Ono što se ne mijenja niti u CLSI ni u EUCAST standardima je granična vrijednost za osjetljivost kod infekcija SŽS-a (MIK≤0.006mg/L). Prema oba standarda parenteralni penicilin se može koristiti kod

pneumonija uzrokovanih sojevima s MIK-om ≤2.0mg/L, s tim da se u EUCAST standardima nalaze i precizne preporuke koje koreliraju MIK-ove i potrebne doze penicilina. Od 2013.g. u EUCAST standardima se izdvaja oralni penicilin za koji ne postoji intermedijarna kategorija koja postoji u CLSI standardima. Epidemiološki gledano u Hrvatskoj nije došlo do značajnijih promjena u osjetljivosti pneumokoka na penicilin, no u 2013.g. sukladno EUCAST standardima ponovno odvojeno prikazujemo osjetljivost na oralni i parenteralni penicilin uz jasniju poruku kliničarima: 31% pneumokoka se ne može liječiti oralnim penicilinom, no parenteralni penicilin je još uvijek lijek izbora u 96% pneumokoknih infekcija koje ne zahvaćaju SŽS, s tim da se kod intermedijarnih izolata (27%) doziranje treba prilagoditi MIK-ovima. Svi intermedijarni izolati će u slučaju Infekcije izvan SŽS reagirati na terapiju parenteralnim penicilinom u dozi od 6 x 2.4g (ili 6 x 4 MIU) za odrasle, a ukoliko je poznat MIK uzročnika može se primijeniti i manja doza.

U izvanbolničkoj sredini prema raznim internacionalnim smjernicama za akutni otitis media i lakšu izvanbolničku pneumoniju najčešće preporučen beta-laktamski antibiotik je amoksicilin. Umjerena i visoka rezistencija pneumokoka na amoksicilin se, također, može odrediti samo određivanjem MIK-ova. EUCAST standardi su donijeli znatno oštrije granične vrijednosti za amoksicilin te prema njima rezistencija pneumokoka na amoksicilin u Hrvatskoj iznosi 4% uz 16% intermedijarnih izolata što ukazuje na potrebu primjene većih doza amoksicilina koje se u pedijatriji već i primjenjuju. Rezistencija pneumokoka na makrolide je visoka i identična prošlogodišnjoj stopi (37%). Visoku stopu rezistencije na makrolide bilježimo još od 2008.g. kad je naglo skočila na 40%. Empirijska uporaba makrolida u liječenju pneumonije ograničena je, stoga, samo na slučajeve jasne sumnje na atipične bakterijske uzročnike. Rezistencija na ko-trimoksazol pokazuje trend pada (43% u 2010.g., 35% u 2011.g., 29% u 2012.g., 27% u 2013.g.), a rezistencija na tetraciklin (26%) je stabilna dugi niz godina. Otpornost pneumokoka na respiratorne kinolone je još uvijek ograničena na sporadične izolate.

Staphylococcus aureus je značajan izvanbolnički pathogen i jedan od najčešćih uzročnika infekcija stečenih tijekom bolničkog liječenja. Rezistencija na meticilin u Europi je još uvijek osobina pretežno bolničkih sojeva iako postaje sve češća i u izvanbolničkih izolata. Od 2010.g. uočljiv je trend smanjenja stope meticilin rezistentnih *Staphylococcus aureus* (MRSA) (25% u 2007. g., 26% u 2008. g., 21% u 2009. g., 16% u 2010. g., 14% u 2011. g., 13% u 2012. g., 12% u 2013.g.). Meticilin osjetljivi stafilokoki (MSSA) su dobro osjetljivi na sve antistafilokokne antibiotike osim penicilina. Zbog već desetljećima visoke rezistencije stafilokoka na penicillin testiranje na ovaj antibiotik nije uključeno u nacionalno praćenje rezistencije, no individualno testiranje je preporučljivo, jer kod rijetkih izolata osjetljivih na penicillin to je još uvijek najdjelotvorniji antistafilokokni antibiotik. Među MRSA sojevima uočen je daljnji pad rezistencije na gentamicin (91% u 2006.g., 81% u 2009.g., 77% u 2010.g., 69% u 2011.g., 64% u 2012.g., 59% u 2013.g.) što bi mogao biti indirektni pokazatelj širenja izvanbolničkih MRSA sojeva. Rezistencija na linezolid i vankomicin nije uočena, a distribucija MIK-ova vankomicina je slična prošlogodišnjoj uz i dalje visok udio sojeva s vrijednošću MIK-a od 2.0 mg/L (20%).

Osjetljivost enterokoka je podjednaka kao prethodne godine osim što je uočen lagani porast vankomicin rezistentnih *E. faecium* (VRE) (1% u 2012.g., 5% u 2013.g.). Ovi sojevi su, međutim, i nadalje ograničeni na pojedine centre i zasada nisu prošireni cijelom Hrvatskom.

Nedostatak djelotvornih antibiotika najviše je uočljiv kod infekcija uzrokovanih multiplorezistentnim gram-negativnim bakterijama. Najčešći uzročnik izvanbolničkih gram-negativnih infekcija je *Escherichia coli* kod koje nije došlo do većih promjena u stopama rezistencije u odnosu na prošlu godinu. Rezistencija na kinolone (14%), ko-trimoksazol (24%) i nitrofurantoin (3%) je identična prošlogodišnjim stopama, a niti udio izolata otpornih na cefalosporine 3. generacije (3% cefepim do 7% cefiksim) se nije bitno promijenio. Do nedavno gotovo isključivi mehanizam rezistencije na 3. generaciju cefalosporina u *E.coli* je bila proizvodnja beta-laktamaza proširenog spektra (engl. "extended spectrum beta-lactamases, ESBL") no sve su učestaliji izolati s plazmidskim AmpC cefalosporinazama. Stope rezistencije se nisu značajnije mijenjale niti u ostalih enterobakterija među kojima su klepsijele i enterobakteri najčešći bolnički patogeni. Rezistencija *K.pneumoniae* na ceftriakson iznosi 33%, a na cefepim 25% što je nešto niže negoli prethodne godine (36% i 28%). Iako se klepsijele koje proizvode karbapenemaze javljaju sve češće, njihov broj u Hrvatskoj još nije tolik da bi se pokazao kao postotak rezistencije na imipenem ili meropenem. Broj enterobaktera koji proizvode karbapenemaze je, međutim, dosegao udio koji po prvi puta u 2013.g. postaje vidljiv kao 1% rezistencije na imipenem i meropenem. Kretanje enterobakterija koje proizvode karbapenemaze je detaljnije opisano u zasebnom poglavlju ove publikacije. Zbog svoje urođene otpornosti na kolistin, tigeciklin te niže osjetljivosti na karbapeneme *Proteus mirabilis* će u budućnosti predstavljati sve veći problem, naročito kod uroloških bolesnika i infekcija povezanih s bolničkom skrbi. Stope rezistencije proteusa na cefalosporine 3. generacije (3% cefepim do 16% ceftriakson, ceftazidim, cefiksim) nisu se značajnije promijenile u odnosu na prethodnu godinu.

U Hrvatskoj i nadalje najveći problem predstavljaju multiplorezistentni nonfermentori *Pseudomonas aeruginosa* i *Acinetobacter baumannii*. Neosjetljivost *P.aeruginosa* na imipenem (17%) i meropenem (18%) je u laganom porastu no u podjednakim stopama prisutna je u Hrvatskoj već dugi niz godina. Najnižu rezistenciju *P.aeruginosa* pokazuje na cefepim (8%) i amikacin (11%). Rezistencija na karbapeneme kod *A. baumannii* se naglo proširila od 2008.g. diljem Hrvatske i u 2013.g. neosjetljivost na imipenem iznosi 79%, a na meropenem 80%. Porasla je i neosjetljivost na ampicilin/sulbaktam (33%). Za razliku od prethodne godine registrirani su i izolati rezistentni na kolistin.

Rezistencija salmonella na ampicilin (10%) identična je prošlogodišnjim vrijednostima. Iako su ESBL sojevi i dalje rijetki među salmonelama, nekoliko centara je prijavilo izolate rezistentne na cefalosporine 3. generacije, što se, meeđutim, nije odrazilo na vidljivi postotak rezistencije na razini Hrvatske. Kao i prošlih godina osjetljivost salmonela na ciprofloksacin na razini Hrvatske je 100%, ali rezistencija na nalidiksičnu kiselinu, koja je bolji pokazatelj niske razine rezistencije na kinolne, je 2%. Rezistencija niskog stupnja može ugroziti ishod liječenja kod sistemnih infekcija te se kod invazivnih izolata mora obavezno određivati MIK za ciprofloksacin i osjetljivima se smatraju samo izolati s MIK ≤0.016mg/L. Rezistencija na ko-trimoksazol je i nadalje niska (3%).

U 2013.g. po prvi puta je uvedeno praćenje rezistencije u *Campylobacter coli* i *Campylobacter jejuni*. U obje vrste rezistencija na ciprofloksacin je iznosila 50%, na eritromicin 1%, a na tetraciklin 27% i 26%.

Tijekom 2013.g. prikupljeno je 27 izolata šigela. Rezistencija na ampicilin je neuobičajeno niska (4%), a na ko-trimoksazol očekivano visoka (89%). Kao i prethodne godine rezistencija nije zabilježena na ko-amoksiklav i cefalosporine 3. generacije, a na kinolone je iznosila 13%.

Stope rezistencije među anaerobnim bakterijama se nisu značajnije mijenjale. Među gram-negativnim anaerobima rezistencija je visoka na penicilin (85%) i klindamicin (27%), a kod gram-pozitivnih anaeroba rezistencija je visoka na metronidazol (50%). Izolati rezistentni na ko-amoksiklav, piperacilin/tazobaktam i ertapenem su rijetki.

DISCUSSION

Due to its high efficacy and narrow spectrum activity penicillin is a first line therapy in streptococcal infections among which sorethroat is the most common one. Macrolides are alternative therapy in patients with hypersensitivity to penicillin. While group A streptococci have not yet been found to be resistant to penicillin, resistance to macrolides is currently 10% which is in range of the rates recorded in the past few years (13% in 2008, 9% in 2009, 8% in 2010, 7% in 2011, and 9% in 2012). Similar to the last year clindamycin results constitutive resistance was 4% and inducible resistance was 1%. In Croatia it is agreed that in streptococci inducible clindamycin resistance is routinely reported as sensitivity to clindamycin with a note that prolonged therapy can lead to resistance.

It is estimated that approx. 10% of upper respiratory tract infections are viral in origin. However, approx. 75% of antibiotic consumption is linked to respiratory infections. The fact that bacteria that normally colonize upper respiratory tract mucosa are at the same time most common respiratory tract pathogens often misleads clinicians and promotes the overuse of antibiotics. Most of the pneumococcal and haemophilus isolates reported in this chapter are from nasopharyngeal swabs and aspirates and therefore are of dubious clinical significance. However, these are isolates representative of microbiota that colonizes upper respiratory tract. Resistance in invasive isolates is described in a separate chapter of this publication. In most international guidelines amoxicillin is the first line therapy in treating acute otitis media due to its high efficacy against pneumococci and *Haemophilus influenzae*. Amoxicillin resistance in *H. influenzae* in 2013 is 17% which is higher than previously reported (9% in 2006, 11% in 2007, 8% in 2008, 10% in 2009, 11% in 2010, 13% in 2011 and 2012). Observed increase of resistance after 2010 can partially be attributed to the introduction of more sensitive EUCAST standards in 2011.

When testing sensitivity to beta-lactam antibiotics in pneumococci commonly used disk diffusion method can only detect non-susceptibility to penicillin. In penicillin non-susceptible isolates high and low level of resistance to penicillin, as well as resistance to other beta-lactams should be determined by performing minimal inhibitory concentration (MICs) which is routinely done for invasive isolates only. Non-susceptibility to penicillin implies that these isolates are not responsive to penicillin therapy only if they are causing central nervous system (CNS) infections but clinicians often consider this to be equal to resistance and are reluctant to use penicillin in any indication. Breakpoint concentrations for beta-lactams have been changing over time both in American (Clinical and Laboratory Standards Institute, CLSI) and European (EUCAST) standards with intention to more accurately define level of resistance clinically relevant in specific clinical indications. One thing that does not change neither in CLSI nor in EUCAST standards is a breakpoint concentration for CNS infections (MIK≤0.006mg/L). According to both standards parenteral penicillin can be used in pneumonia caused by strains with MICs ≤2.0mg/L and furthermore EUCAST standards provide detailed correlation of MICs and penicillin dosing. Since 2013 EUCAST standards separately report oral

penicillin for which there is no intermediate category which is different from CLSI standards. From epidemiological point of view there have been no changes in resistance of pneumococci but following EUCAST standards in 2013 we are reporting again oral penicillin separately from parenteral penicillin with a clear message to clinicians: 31% of pneumococci cannot be treated with oral penicillin but parenteral penicillin is still a drug of choice in 96% of pneumococcal infections that do not affect CNS. However, in intermediate isolates (27%) dosing should be adjusted to penicillin MICs. In infections not affecting CNS all penicillin intermediate isolates will respond to treatment with parenteral penicillin if a dose of 6 x 2.4g (or 6 x 4 MIU) is used for adults. If penicillin MIC of the causative organism is known dosing can be lowered accordingly.

According to different international guidelines a beta-lactam most commonly used in ambulatory care for acute otitis media or less severe community acquired pneumonia is amoxicillin. Intermediate and high level resistance to amoxicillin can also be detected only by determining MICs. Amoxicillin breakpoints are significantly lower in EUCAST standards so that resistance of pneumococci in Croatia is 4% and intermediate resistance is 16% which indicates the need for higher dosing of amoxicillin, a practice that is already common in paediatrics. Resistance of pneumococci to macrolides is high and identical to the last year rate (37%). Macrolide resistance rate is high since 2008 after a sudden increase to 40%. Empirical use of macrolides in treatment of pneumonia is therefore restricted to clear cases of atypical infection. Resistance to co-trimoxazole is showing a decreasing trend (43% in 2010, 35% in 2011, 29% in 2012, 27% in 2013), and tetracycline resistance (26%) is stable for many years. Quinolone resistance is still recorded in sporadic pneumococcal isolates only.

Staphylococcus aureus is a respectable community acquired pathogen and one of the most frequent pathogens causing health care associated infections. In Europe resistance to methicillin is still predominantly a feature of nosocomial strains although methicillin resistant isolates are becoming increasingly prevalent in the community as well. The rate of methicillin resistant *Staphylococcus aureus* (MRSA) started to decrease in 2010 (25% in 2007, 26% in 2008, 21% in 2009, 16% in 2010, 14% in 2011, 13% in 2012, and 12% in 2013). Methicillin sensitive staphylococci (MSSA) are highly sensitive to all antibiotics except penicillin. As high penicillin resistance is present for decades penicillin testing is not included in national surveillance but individual testing is recommended because for rare penicillin susceptible isolates penicillin is still the most active antistaphylococcal drug. Among MRSA isolates there is a further decrease in gentamicin resistance (91% in 2006, 81% in 2009, 77% in 2010, 69% in 2011, 64% in 2012, and 59% in 2013) which may be an indirect indicator of the spread of community acquired MRSA strains. Resistance to linezolid and vancomycin was not recorded and vancomycin MIC distribution is similar to the last year results with high rate of isolates showing MIC of 2.0 mg/L (20%).

Sensitivity of enterococci is similar as reported last year except for the slight increase in vancomycin resistant *E.faecium* (VRE) strains (1% in 2012, 5% in 2013). These isolates, however, continue to be recorded in few centres only and as for now are not spread throughout the country.

Lack of effective antibiotics is most visible in infections caused by gram-negative bacteria. The most frequent causative agent of community acquired gram-negative infections is *Escherichia coli* whose resistance rates did not change much as compared with the previous year. Resistance rates to quinolones (14%), co-trimoxzole (24%) and nitrofurantoin (3%) are identical to last year rates and resistance to 3rd generation cephalosporins (3% cefepime to 7% cefixime) did not change significantly. Until recently resistance to 3rd generation cephalosporins in *E.coli* was almost exclusively mediated by production of extended spectrum beta-lactamases (ESBL) but plasmid mediated AmpC cephalosporinases are becoming more frequent. Other enterobacteriaceae, among which klebsiellas and enterobacters are the most common nosocomial pathogens, did not significantly change resistance rates either. Ceftriaxone resistance in *K.pneumoniae* is 33%, and cefepime resistance is 25% which is somewhat lower than the last year (36% and 28%). Although carbapenemase producing klebsiellae are being increasingly reported in Croatia their number is not that high to be seen as a percentage of resistance to imipenem or meropenem. The number of carbapenemase producing enterobacters, however, has for the first time reached the rate visible as 1% resistance to imipenem and meropenem. The spread of carbapenemase producing enterobacteriaceae is described in more details in a separate chapter of this publication. Due to its innate resistance to colistin, tygecycline and low sensitivity to carbapenems *Proteus mirabilis* will pose a growing problem in the future, especially in urology patients and in health care associated infections. Resistance rates to 3rd generation cephalosporins (3% cefepime to 16% ceftriaxone, ceftazidime, and cefixime) did not change significantly as compared to the last year.

Multiply resistant nonfermentative bacteria, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* still present the major problem in Croatia. Non-susceptibility of *P.aeruginosa* to imipenem (17%) and meropenem (18%) is slightly increasing although it has been present at similar levels for many years in Croatia. Lowest resistance rates are recorded cefepime (8%) and amikacin (11%). Carbapenem resistance in *A. baumannii* has rapidly spread throughout Croatia since 2008 and in 2013 non-susceptibility to imipenem is 79%, and to meropenem 80%. Non-susceptibility to ampicillin/sulbactam (33%) is also increasing. Unlike last year this year isolates resistant to colistin were also recorded.

Ampicillin resistance in salmonellae (10%) is identical to the last year rates. Although ESBL isolates are still rare among salmonellae a few centres reported isolates resistant to 3rd generation cephalosporins which is still not visible as a resistance rate at the national level. As in the previous years susceptibility of salmonellae to ciprofloxacin is 100% but resistance to nalidixic acid, which is an indicator of low level resistance to quinolones, is 2%. Low level quinolone resistance can jeopardize treatment of systemic infections and therefore ciprofloxacin MICs should be determined in all invasive isolates. Only isolates with MIC $\leq$0.016mg/L are considered fully susceptible. Resistance to co-trimoxazole is still low (3%).

Campylobacter coli and *Campylobacter jejuni* were for the first time included in surveillance in 2013. In both species resistance to ciprofloxacin was 50%, to erythromycin 1%, and to tetracycline 27% and 26%.

During 2013 altogether 27 shigella isolates were collected. Resistance to ampicillin was unusually low (4%), and to co-trimoxazole expectedly high (89%). The same as during the previous year no resistance was recorded for co-amoxiclav and 3rd generation cephalosporins and resistance to quinolones was 13%.

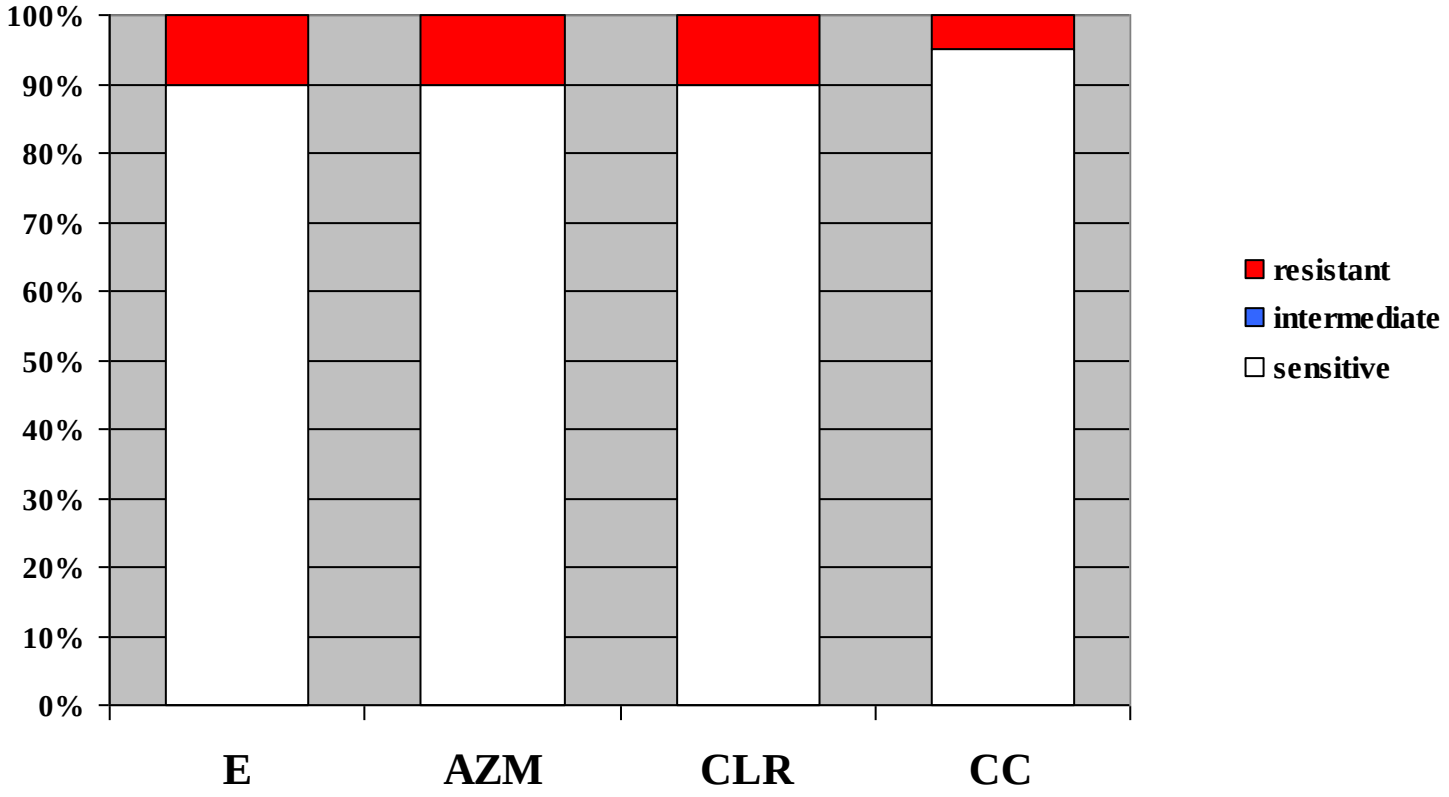
Resistance rates among anaerobic bacteria did not change much. High rates of resistance to penicillin (85%) and clindamycin (27%) were recorded in gram-negative anaerobes, and in gram-positive anaerobes resistance was high to metronidazol (50%). Isolates resistant to co-amoxiclav, piperacillin/tazobactam and ertapenem were only rarely reported.

ANTIBIOTICI / ANTIBIOTICS:

$P_{parenteral}$	penicillin parenteral
P_{oral}	penicillin oral
AMP	ampicillin
AMC	amoxicillin + clavulanic acid
SAM	ampicillin + sulbactam
FOX	cefoxitin / methicillin
CN	cefalexin (I. gen. cephalosporins)
CXM	cefuroxime (II. gen. cephalosporins)
$CXM_{i.v.}$	cefuroxime parenteral
CXM_{oral}	cefuroxime oral
CAZ	ceftazidime (III. gen. cephalosporins)
CRO	ceftriaxone (III. gen. cephalosporins)
CTB	ceftibuten (III. gen. cephalosporins)
CFM	cefixime (III. gen. cephalosporins)
CFEP	cefepime (IV. gen. cephalosporins)
PTZ	piperacillin/tazobactam
ERT	ertapenem
IMP	imipenem
MER	meropenem
E	erythromycin
AZM	azithromycin
CLR	clarythromycin
CC	clindamycin
TE	tetracycline
SXT	co-trimoxazole
NF	nitrofurantoin
VA	vancomycin
RIF	rifampicin
CIP	ciprofloxacin
NOR	norfloxacin
GM	gentamicin
NT	netilmicin
AN	amikacin
MUP	mupirocin
MTZ	metronidazole
MOX	moxifloxacin
LZD	linezolid
NA	nalidixic acid
COL	colistin
TGC	tigecycline

UK = ukupan broj izolata / *total number of isolates*
No = broj izolata / *number of isolates*
I% = % intermedijarnih izolata / % *of intermediate isolates*
R% = % rezistentnih izolata / % *of resistant isolates*

Beta-hemolitički streptokok grupe A
Group A beta-hemolytic streptococcus
(1.01. - 31.12. 2013.) **- osjetljivost na antibiotike u RH**
- sensitivity to antibiotics in Croatia



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Croatian Committee for Antibiotic Resistance Surveillance

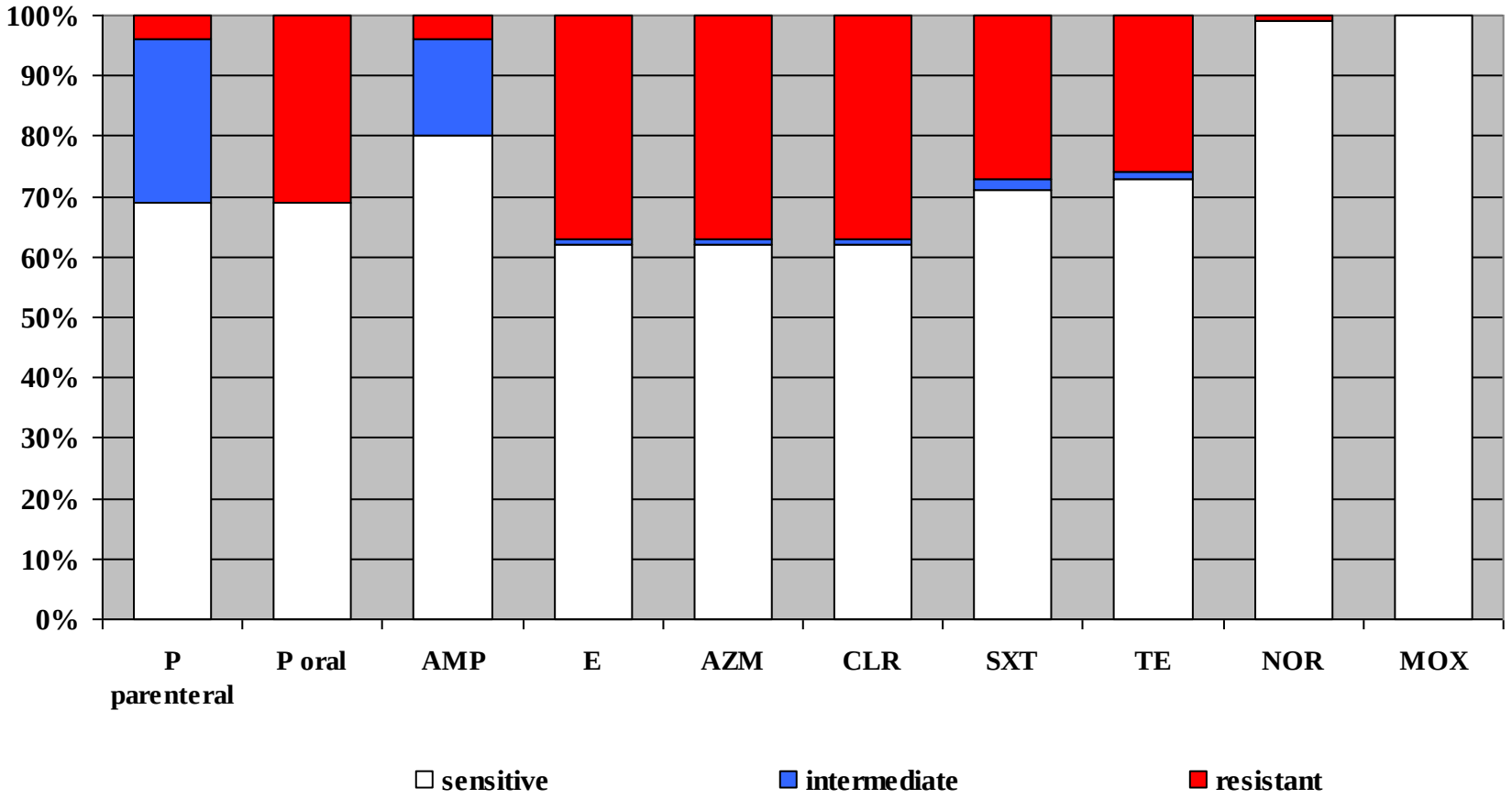
Beta-hemolitički streptokok grupe A
Group A streptococcus

- rezistencija na antibiotike u razdoblju od 1.01.- 31.12. 2013.
  zbirni prikaz izolata iz 40 centra u RH
- antibiotic resistance for the period 1.01. - 31.12. 2013.
  summary results for the isolates from 40 centers in Croatia

ANTIBIOTIK ANTIBIOTIC	Broj izolata No. of isolates	% rezistentnih (% intermedijarnih) izolata % of resistant (% of intermediate) isolates	Raspon lokalnih rezultata* Range of local results*
Erythromycin	12 993	10 (0)	1 (0) - 22 (0)
Azithromycin	12 993	10 (0)	1 (0) - 22 (0)
Clarythromycin	12 993	10 (0)	1 (0) -22 (0)
Clindamycin	12 986	5 (0)	
constitutive		4	0 - 20
inducible		1	0 - 4

* rezultati centara s malim brojem izolata (<30) nisu uzeti u obzir
results from the centers with small number of isolates (<30) were not taken into consideration

Streptococcus pneumoniae (1.10. - 31.12. 2013.) - osjetljivost na antibiotike u RH - sensitivity to antibiotics in Croatia



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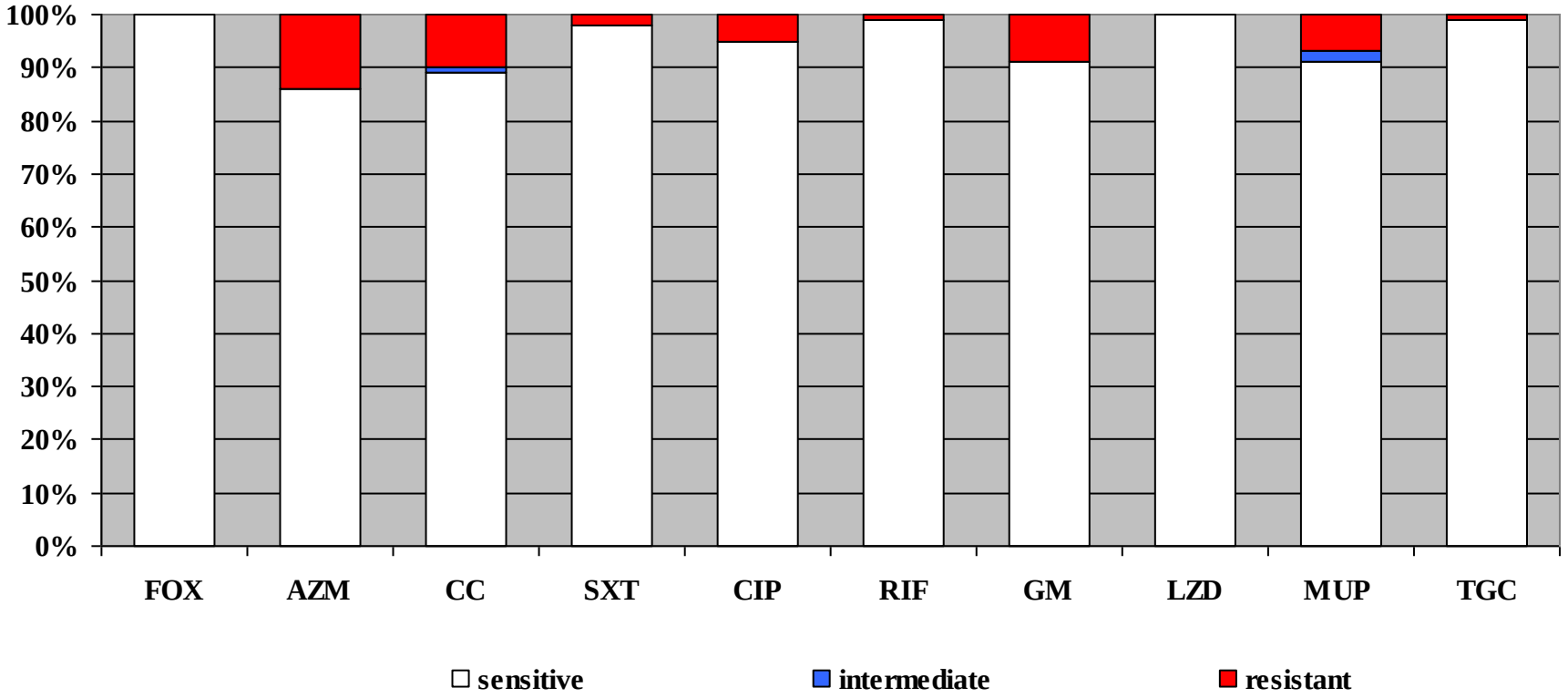
Streptococcus pneumoniae

- rezistencija na antibiotike u razdoblju od 1.10.- 31.12. 2013.
zbirni prikaz izolata iz 40 centra u RH
- antibiotic resistance for the period 1.10. - 31.12. 2013.
summary results for the isolates from 40 centers in Croatia

ANTIBIOTIK ANTIBIOTIC	Broj izolata No. of isolates	% rezistentnih (% intermedijarnih) izolata % of resistant (% of intermediate) isolates	Raspon lokalnih rezultata* Range of local results*
Penicillin parenteral	2 543	4 (27)	0 (0) - 28 (21)
Penicilin oral	2 543	31 (0)	0 (0) - 50 (0)
Ampicillin	1 921	4 (16)	0 (0) - 18 (8)
Erythromycin	2 643	37 (1)	11 (0) - 54 (4)
Azithromycin	2 643	37 (1)	11 (0) - 54 (4)
Clarythromycin	2 643	37 (1)	11 (0) - 54 (4)
Co-trimoxazole	2 639	27 (2)	2 (0) - 47 (0)
Tetracycline	2 388	26 (1)	0 (0) - 52 (0)
Norfloxacin	2 535	1 (0)	0 (0) - 15 (0)
Moxifloxacin	2 493	0 (0)	0 (0) - 8 (0)

* rezultati centara s malim brojem izolata (<30) nisu uzeti u obzir
results from the centers with small number of isolates (<30) were not taken into consideration

Staphylococcus aureus MSSA (1.10. - 31.12. 2013.) - osjetljivost na antibiotike u RH - sensitivity to antibiotics in Croatia



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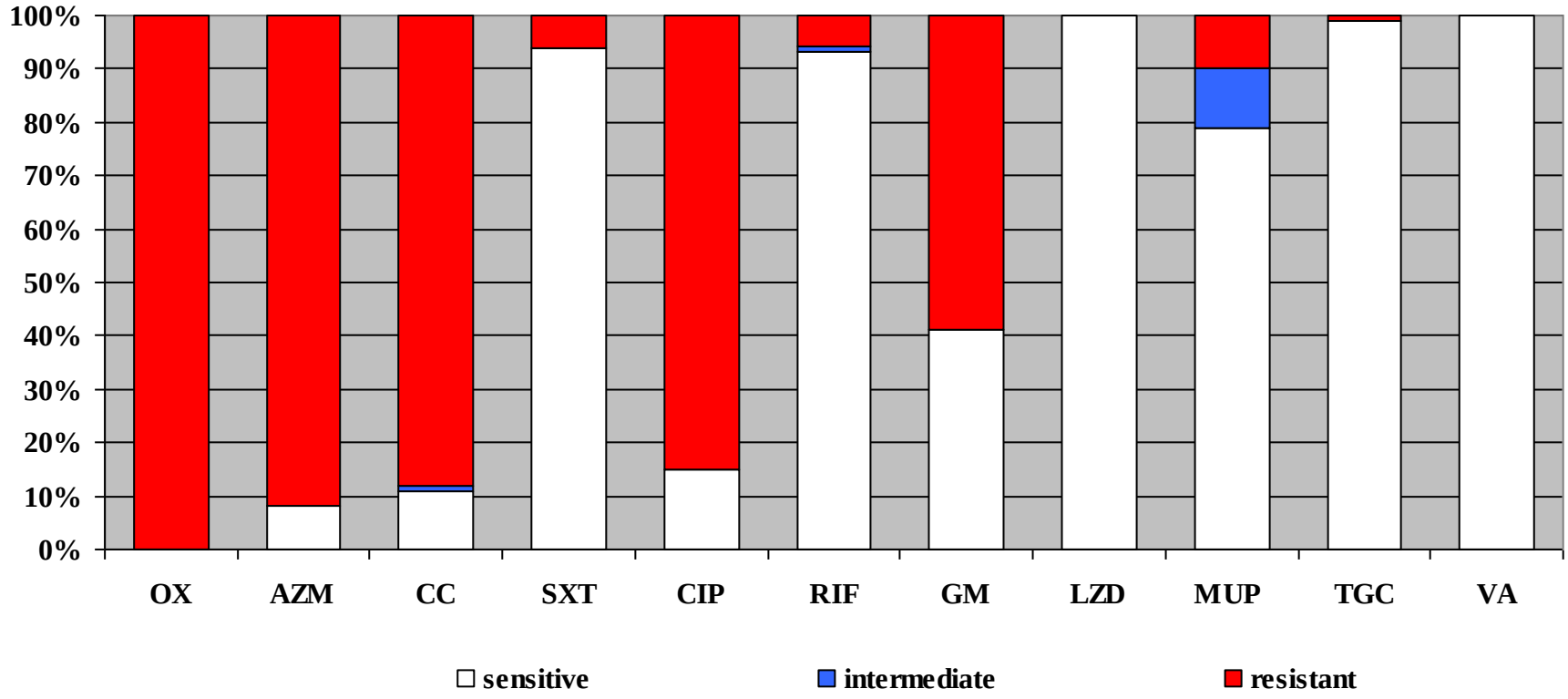
Staphylococcus aureus / MSSA

- rezistencija na antibiotike u razdoblju od 1.10.- 31.12. 2013.
zbirni prikaz izolata iz 40 centra u RH
- antibiotic resistance for the period 1.10. - 31.12. 2013.
summary results for the isolates from 40 centers in Croatia

ANTIBIOTIK ANTIBIOTIC	Broj izolata No. of isolates	% rezistentnih (% intermedijarnih) izolata % of resistant (% of intermediate) isolates	Raspon lokalnih rezultata* Range of local results*
Cefoxitin / Methicillin	4 041	0 (0)	0 (0) - 0 (0)
Azithromycin	3 899	14 (0)	3 (0) - 33 (0)
Clindamycin	3 898	10 (1)	2 (0) - 25 0
Co-trimoxazole	3 905	2 (0)	0 (0) - 15 (0)
Ciprofloxacin	3 875	5 (0)	0 (0) - 18 (0)
Rifampicin	3 276	1 (0)	0 (0) - 8 (1)
Gentamicin	3 885	9 (0)	0 (0) - 19 (0)
Linezolid	3 377	0 (0)	0 (0) – 5 (0)
Mupirocin	3 549	7 (2)	0 (0) - 22 (0)
Tigecycline	3 232	1 (0)	0 (0) - 19 (0)

* rezultati centara s malim brojem izolata (<30) nisu uzeti u obzir
results from the centers with small number of isolates (<30) were not taken into consideration

Staphylococcus aureus MRSA (1.10. - 31.12. 2013.) - osjetljivost na antibiotike u RH - sensitivity to antibiotics in Croatia



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Staphylococcus aureus / MRSA

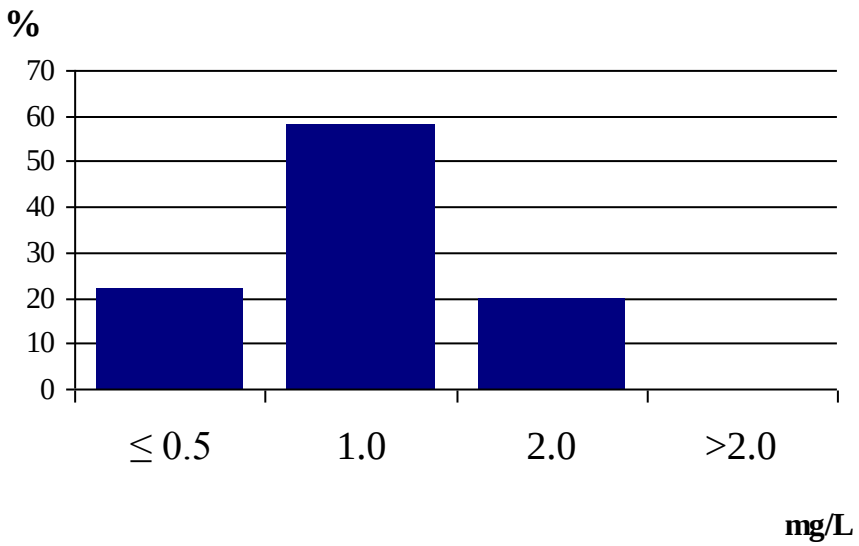
- rezistencija na antibiotike u razdoblju od 1.10.- 31.12. 2013.
zbirni prikaz izolata iz 40 centra u RH
- antibiotic resistance for the period 1.10. - 31.12. 2013.
summary results for the isolates from 40 centers in Croatia

ANTIBIOTIK ANTIBIOTIC	Broj izolata No. of isolates	% rezistentnih (% intermedijarnih) izolata % of resistant (% of intermediate) isolates	Raspon lokalnih rezultata* Range of local results*
Cefoxitin / Methicillin	573	100 (0)	100 (0) - 100 (0)
Azithromycin	570	92 (0)	89 (0) - 98 (0)
Clindamycin	567	88 (1)	81 (0) - 98 (0)
Co-trimoxazole	570	6 (0)	0 (0) - 16 (0)
Ciprofloxacin	565	85 (0)	84 (0) - 98 (0)
Rifampicin	539	6 (1)	0 (0) - 8 (0)
Gentamicin	572	59 (0)	42 (0) - 84 (0)
Linezolid	546	0 (0)	0 (0) - 0 (0)
Mupirocin	502	10 (11)	0 (35) - 13 (3)
Tigecycline	478	1 (0)	0 (0) -0 (0)
Vankomycin	422	0 (0)	0 (0) - 0 (0)

* rezultati centara s malim brojem izolata (<30) nisu uzeti u obzir
results from the centers with small number of isolates (<30) were not taken into consideration

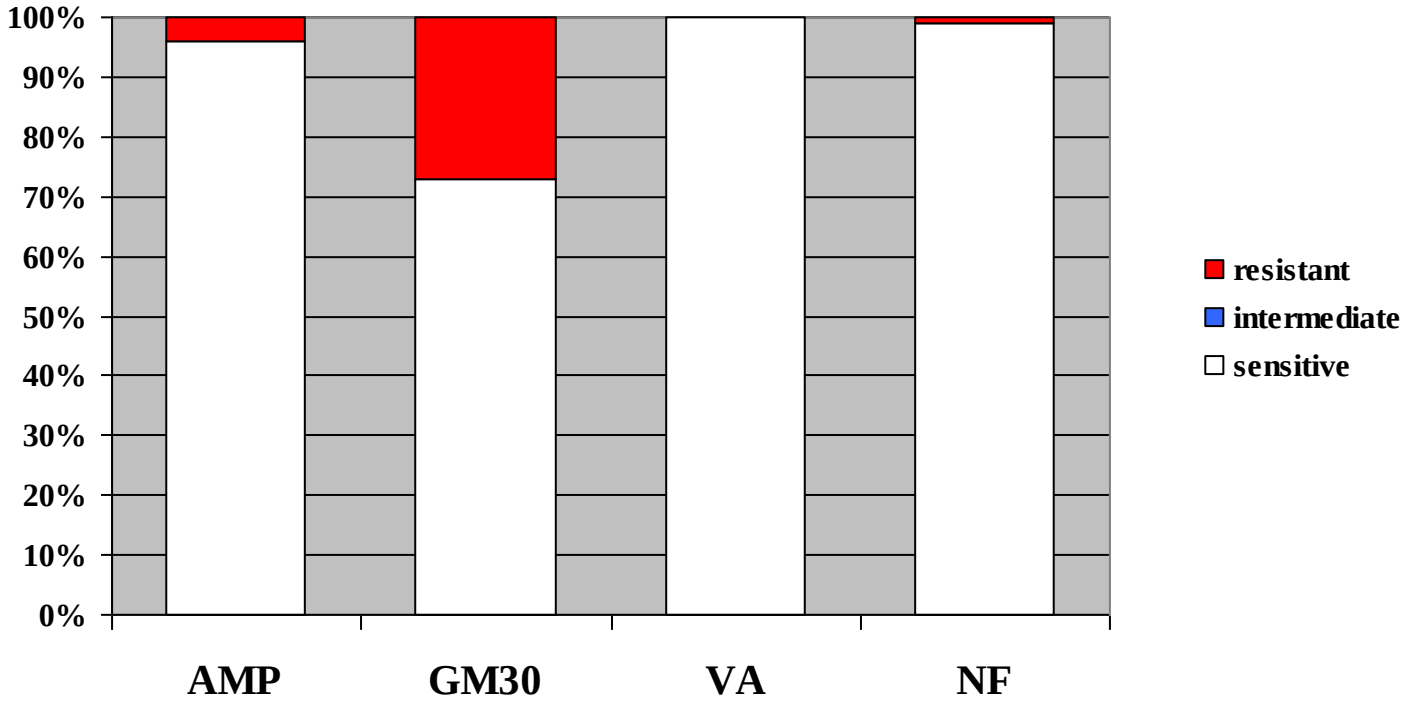
Staphylococcus aureus : MRSA

Distribucija MIK-ova vankomicina, (422 MRSA izolata), 1.10. – 31.12. 2013.
Vancomycin MIC distribution, (422 MRSA isolates), 1.10. – 31.12. 2013.



MIK = minimalna inhibitorna koncentracija
MIC = minimal inhibitory concentration

Enterococcus faecalis (1.10. - 31.12. 2013.) - osjetljivost na antibiotike u RH - sensitivity to antibiotics in Croatia



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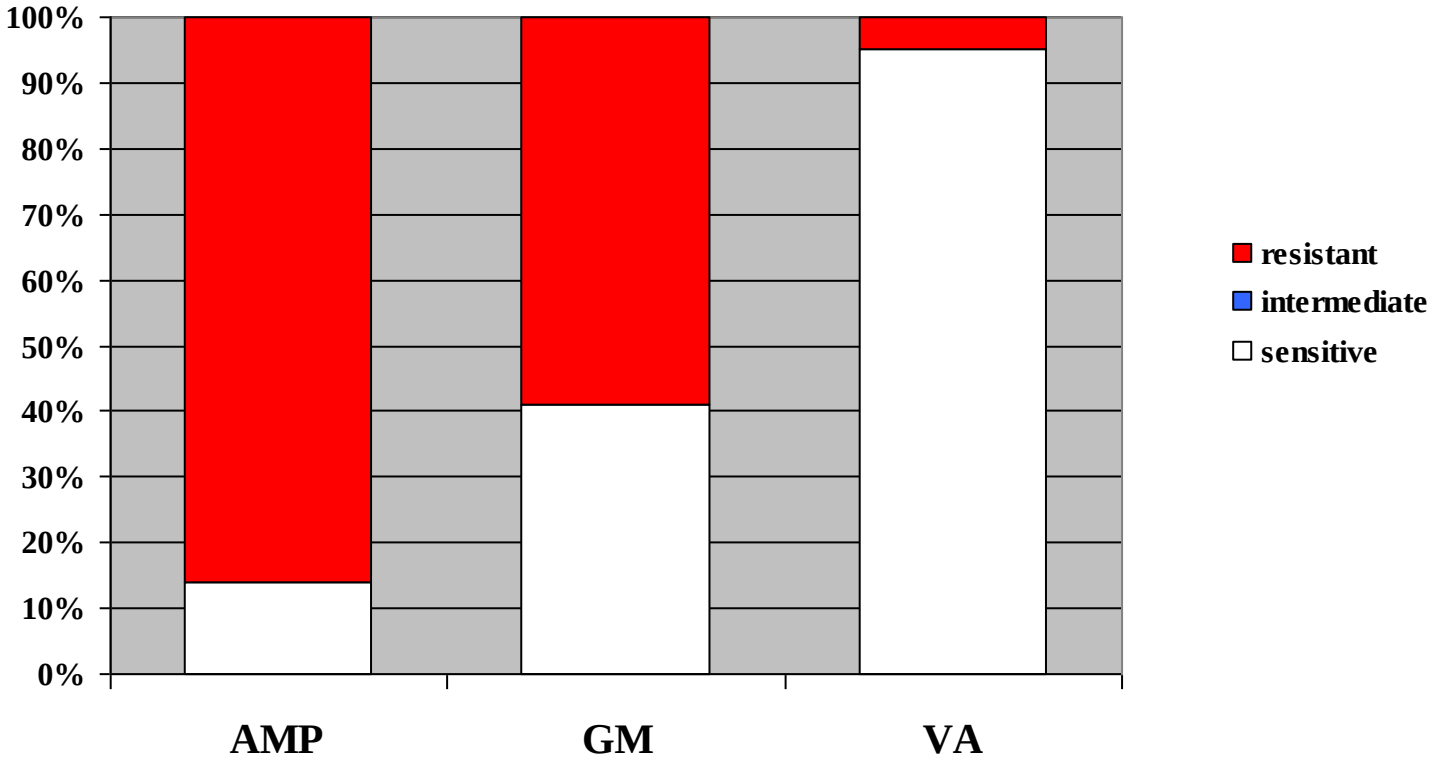
Enterococcus faecalis

- rezistencija na antibiotike u razdoblju od 1.10.- 31.12. 2013.
zbirni prikaz izolata iz 40 centra u RH
- antibiotic resistance for the period 1.10. - 31.12. 2013.
summary results for the isolates from 40centers in Croatia

ANTIBIOTIK ANTIBIOTIC	Broj izolata No. of isolates	% rezistentnih (% intermedijarnih) izolata % of resistant (% of intermediate) isolates	Raspon lokalnih rezultata* Range of local results*
Ampicillin	5 554	4 (0)	0 (0) - 24 (0)
Gentamicin	5 030	27 (0)	0 (0) - 60 (0)
Vancomycin	5 028	0 (0)	0 (0) - 1 (0)
Nitrofurantoin	4 915	1 (0)	0 (0) - 14 (0)

* rezultati centara s malim brojem izolata (<30) nisu uzeti u obzir
results from the centers with small number of isolates (<30) were not taken into consideration

Enterococcus faecium (1.10. - 31.12. 2013.) - osjetljivost na antibiotike u RH - sensitivity to antibiotics in Croatia



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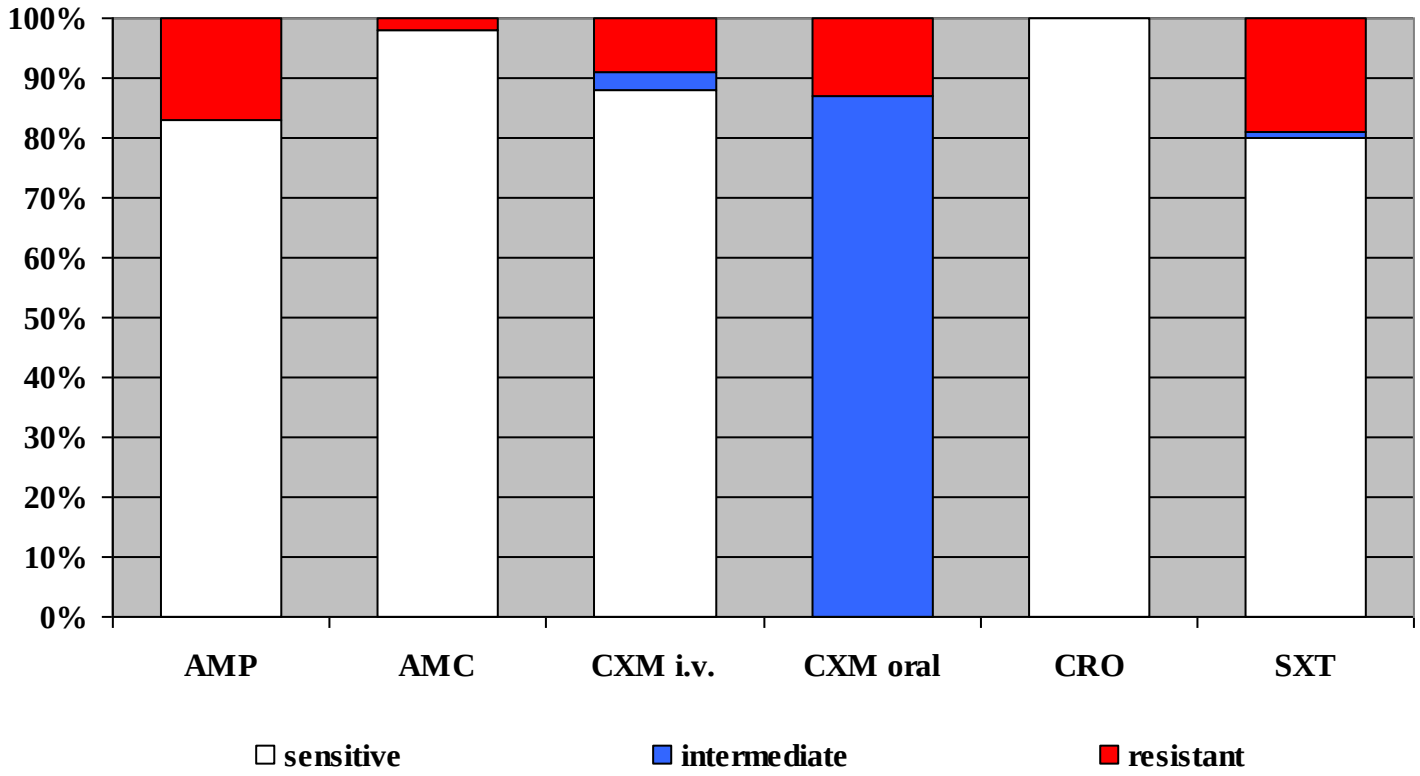
Enterococcus faecium

- rezistencija na antibiotike u razdoblju od 1.10.- 31.12. 2013.
zbirni prikaz izolata iz 40 centra u RH
- antibiotic resistance for the period 1.10. - 31.12. 2013.
summary results for the isolates from 40 centers in Croatia

ANTIBIOTIK ANTIBIOTIC	Broj izolata No. of isolates	% rezistentnih (% intermedijarnih) izolata % of resistant (% of intermediate) isolates	Raspon lokalnih rezultata* Range of local results*
Ampicillin	535	86 (0)	51 (0) - 100 (0)
Gentamicin	535	59 (0)	14 (0) - 73 (0)
Vancomycin	659	5 (0)	0 (0) - 20 (0)

* rezultati centara s malim brojem izolata (<30) nisu uzeti u obzir
results from the centers with small number of isolates (<30) were not taken into consideration

Haemophilus influenzae (1.10. - 31.12. 2013.) - osjetljivost na antibiotike u RH - sensitivity to antibiotics in Croatia



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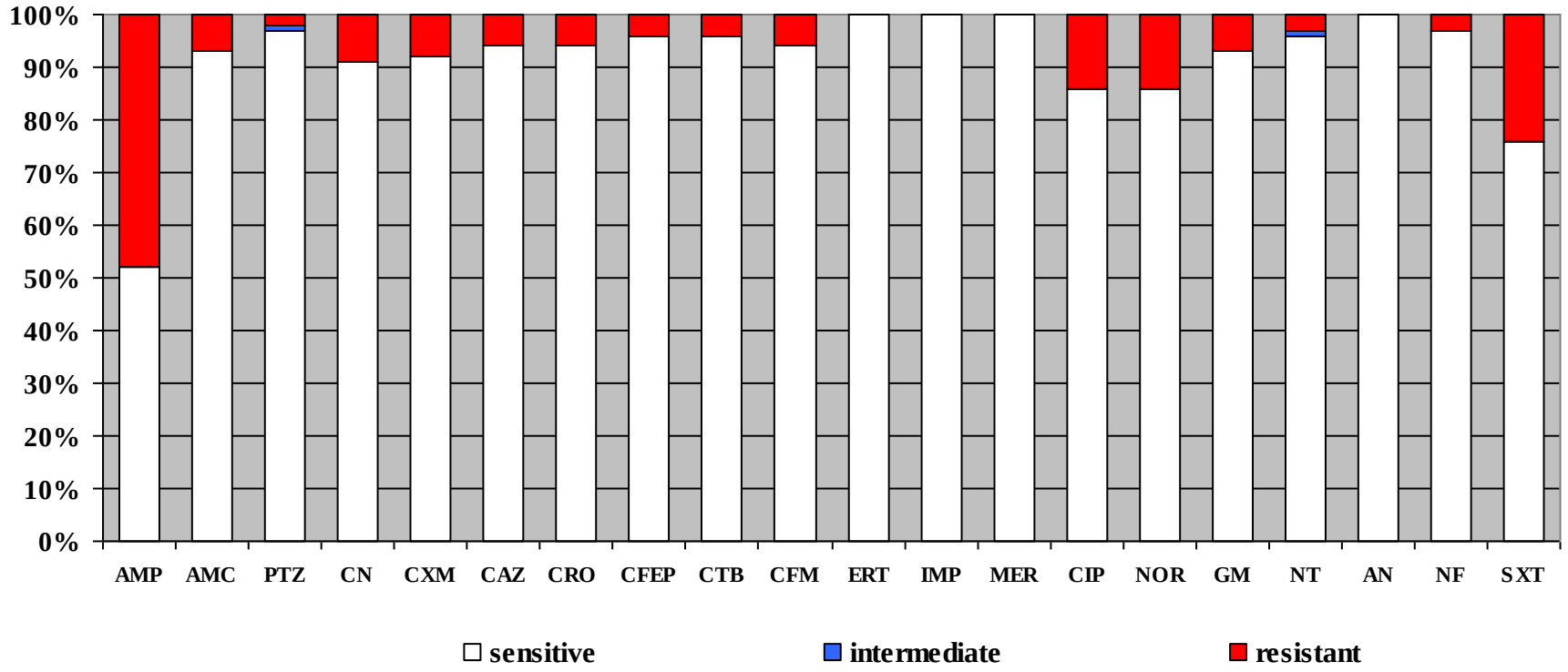
Haemophilus influenzae

- rezistencija na antibiotike u razdoblju od 1.10.- 31.12. 2013.
zbirni prikaz izolata iz 40 centra u RH
- antibiotic resistance for the period 1.10. - 31.12. 2013.
summary results for the isolates from 40 centers in Croatia

ANTIBIOTIK **ANTIBIOTIC**	**Broj izolata** **No. of isolates**	**% rezistentnih** **(% intermedijarnih) izolata** **% of resistant** **(% of intermediate) isolates**	**Raspon lokalnih rezultata*** **Range of local results***
Ampicillin	2 030	17 (0)	5 (0) - 65 (0)
Amoxicillin + clav. acid	2 030	2 (0)	0 (0) - 17 (0)
Cefuroxime $_{\textbf{i.v.}}$	1 889	9 (3)	0 (0) - 36 (0)
Cefuroxime $_{\textbf{oral}}$	1 845	13 (87)	0 (100) - 37 (63)
Ceftriaxone	1 944	0 (0)	0 (0) -0 (0)
Co-trimoxazole	2 088	19 (1)	6 (0) - 51 (0)

* rezultati centara s malim brojem izolata (<30) nisu uzeti u obzir
results from the centers with small number of isolates (<30) were not taken into consideration

Escherichia coli (1.10. - 31.12. 2013.) - osjetljivost na antibiotike u RH - sensitivity to antibiotics in Croatia



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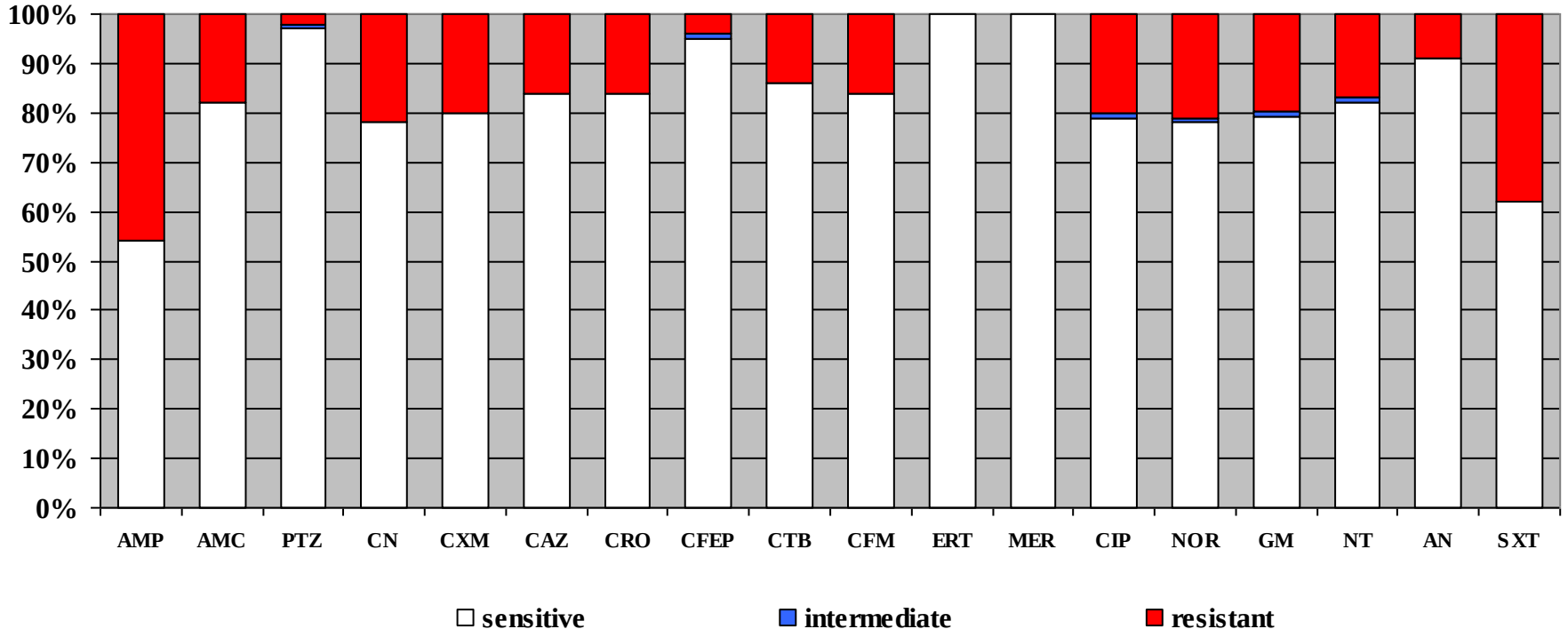
Escherichia coli

- rezistencija na antibiotike u razdoblju od 1.10.- 31.12. 2013.
zbirni prikaz izolata iz 40 centra u RH
- antibiotic resistance for the period 1.10. - 31.12. 2013.
summary results for the isolates from 40 centers in Croatia

ANTIBIOTIK ANTIBIOTIC	Broj izolata No. of isolates	% rezistentnih (% intermedijarnih) izolata % of resistant (% of intermediate) isolates	Raspon lokalnih rezultata* Range of local results*
Ampicillin	18 346	48 (0)	29 (0) - 69 (0)
Amoxicillin + clav. acid	18 352	7 (0)	2 (0) - 15 (0)
Piperacillin + tazobactam	18 246	2 (1)	0 (0) - 8 (1)
Cephalexin	17 899	9 (0)	4 (0) - 22 (0)
Cefuroxime	18 282	8 (0)	2 (0) - 17 (0)
Ceftazidime	18 343	6 (0)	1 (0) - 15 (2)
Ceftriaxone	18 353	6 (0)	1 (0) - 16 (2)
Cefepime	18 236	3 (0)	0 (0) - 10 (1)
Ceftibuten	17 943	4 (0)	1 (0) - 17 (0)
Cefixime	17 938	7 (0)	2 (0) - 17 (0)
Ertapenem	18 229	0 (0)	0 (0) - 1 (0)
Imipenem	18 278	0(0)	0 (0) - 0 (0)
Meropenem	18 266	0 (0)	0 (0) - 1 (0)
Ciprofloxacin	18 351	14 (0)	6 (1) - 24 (1)
Norfloxacin	18 194	14 (0)	6 (1) - 24 (2)
Gentamicin	18 323	6 (0)	3 (0) - 17 (2)
Netilmicin	17 889	3 (1)	0 (0) - 7 (0)
Amikacin	18 080	0 (0)	0 (0) - 2 (0)
Nitrofurantoin	17 930	3 (0)	0 (0) - 9 (0)
Co-trimoxazole	18 342	24 (0)	12 (0) - 40 (0)

* rezultati centara s malim brojem izolata (<30) nisu uzeti u obzir
results from the centers with small number of isolates (<30) were not taken into consideration

Proteus mirabilis (1.10. - 31.12. 2013.) - osjetljivost na antibiotike u RH - sensitivity to antibiotics in Croatia



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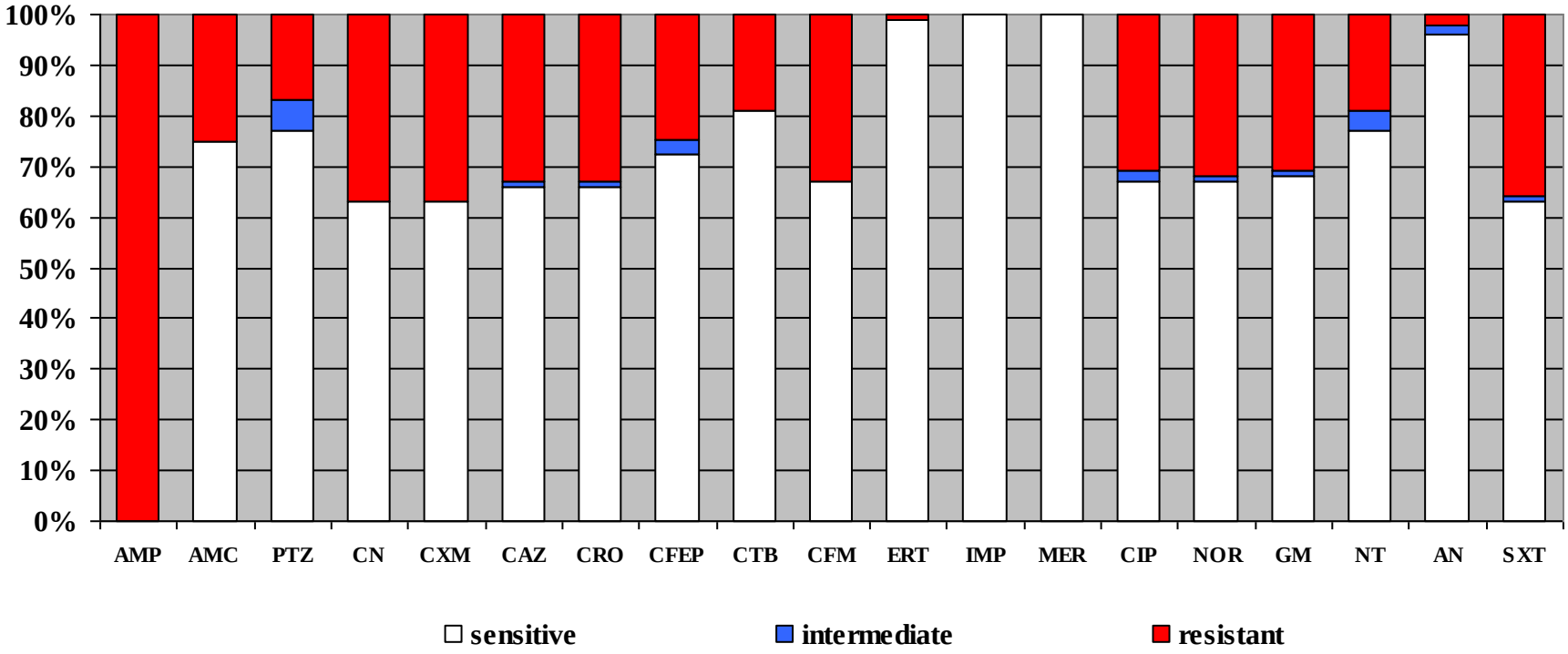
Proteus mirabilis

- rezistencija na antibiotike u razdoblju od 1.10.- 31.12. 2013.
zbirni prikaz izolata iz 40 centra u RH
- antibiotic resistance for the period 1.10. - 31.12. 2013.
summary results for the isolates from 40 centers in Croatia

ANTIBIOTIK ANTIBIOTIC	Broj izolata No. of isolates	% rezistentnih (% intermedijarnih) izolata % of resistant (% of intermediate) isolates	Raspon lokalnih rezultata* Range of local results*
Ampicillin	3 941	46 (0)	17 (0) - 100 (0)
Amoxicillin + clav. acid	3 941	18 (0)	4 (0) - 38 (0)
Piperacillin + tazobactam	3 917	2 (1)	0(0) - 16 (7)
Cephalexin	3 844	22 (0)	4 (0) - 48 (0)
Cefuroxime	3 936	20 (0)	2 (0) - 45 (0)
Ceftazidime	3 940	16 (0)	1 (0) - 42 (0)
Ceftriaxone	3 941	16 (0)	1 (0) - 43 (0)
Cefepime	3 917	3 (1)	0 (0) - 23 (0)
Ceftibuten	3 800	14 (0)	1 (0) - 42 (0)
Cefixime	3 772	16 (0)	0 (0) - 45 (0)
Ertapenem	3 915	0 (0)	0 (0) - 4 (0)
Meropenem	3 918	0 (0)	0 (0) - 1 (1)
Ciprofloxacin	3 939	20 (1)	0 (0) - 54 (0)
Norfloxacin	3 899	21 (1)	3 (0) - 60 (2)
Gentamicin	3 941	20 (1)	5 (0) - 48 (2)
Netilmicin	3 811	17 (1)	0 (0) - 47 (0)
Amikacin	3 888	9 (0)	0 (0) - 36 (0)
Co-trimoxazole	3 939	38 (0)	18 (0) - 65 (0)

* rezultati centara s malim brojem izolata (<30) nisu uzeti u obzir
results from the centers with small number of isolates (<30) were not taken into consideration

Klebsiella pneumoniae (1.10. - 31.12. 2013.) - osjetljivost na antibiotike u RH - sensitivity to antibiotics in Croatia



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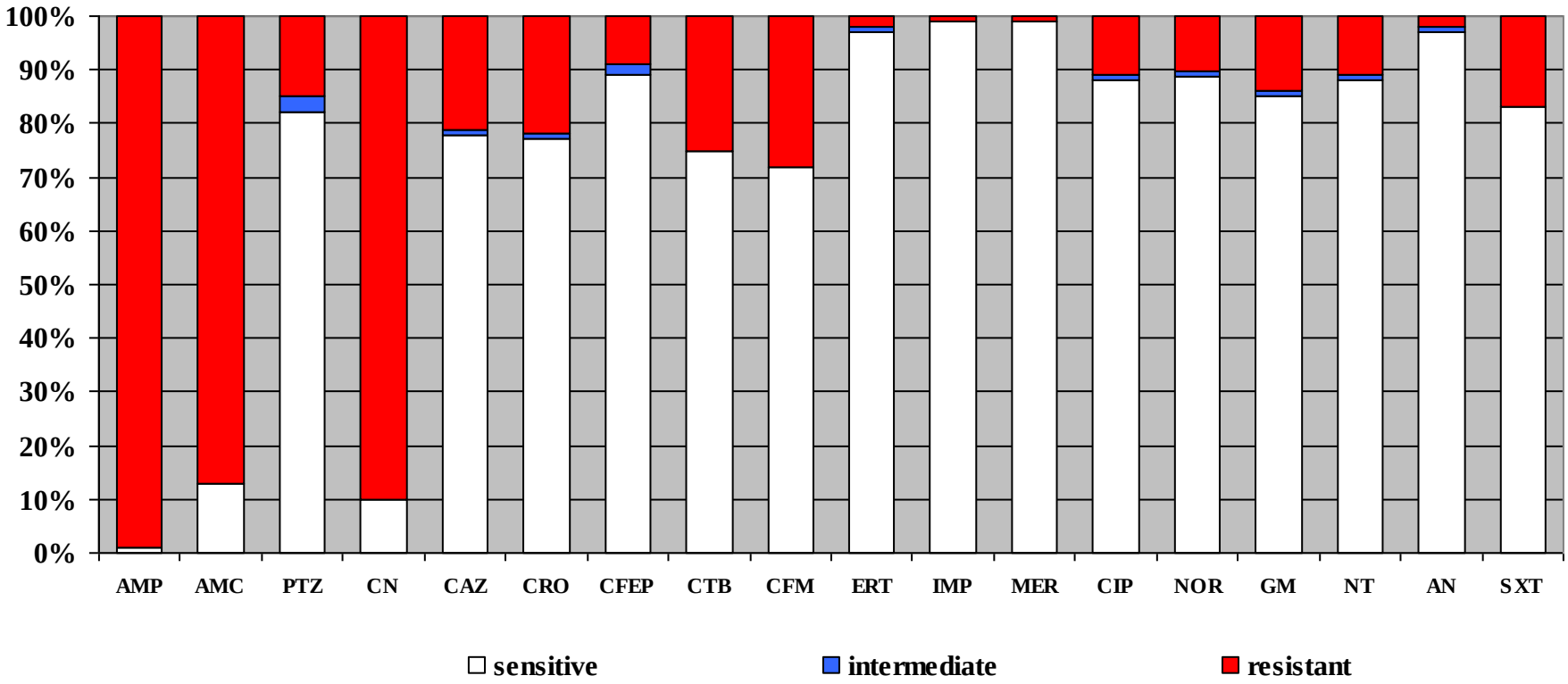
Klebsiella pneumoniae

- rezistencija na antibiotike u razdoblju od 1.10.- 31.12. 2013.
zbirni prikaz izolata iz 40 centra u RH
- antibiotic resistance for the period 1.10. - 31.12. 2013.
summary results for the isolates from 40 centers in Croatia

ANTIBIOTIK ANTIBIOTIC	Broj izolata No. of isolates	% rezistentnih (% intermedijarnih) izolata % of resistant (% of intermediate) isolates	Raspon lokalnih rezultata* Range of local results*
Ampicillin	4 295	100 (0)	94 (0) - 100 (0)
Amoxicillin + clav. acid	4 300	25 (0)	7 (0) - 61 (0)
Piperacillin + tazobactam	4 261	17 (6)	0 (0) - 40 (7)
Cephalexin	4 131	37 (0)	18 (0) - 59 (0)
Cefuroxime	4 296	37 (0)	15 (0) - 63 (0)
Ceftazidime	4 298	33 (1)	9 (0) - 54 (7)
Ceftriaxone	4 299	33 (1)	9 (0) - 62 (0)
Cefepime	4 258	25 (3)	0 (0) - 47 (16)
Ceftibuten	4 140	19 (0)	0 (0) - 44 (0)
Cefixime	4 127	33 (0)	12 (0) - 62 (0)
Ertapenem	4 250	1 (0)	0 (0) - 13 (1)
Imipenem	4 276	0 (0)	0 (0) - 1 (0)
Meropenem	4 273	0 (0)	0 (0) - 2 (0)
Ciprofloxacin	4 293	31 (2)	9 (0) - 62 (1)
Norfloxacin	4 214	32 (1)	9 (0) - 68 (0)
Gentamicin	4 296	31 (1)	7 (1) - 54 (0)
Netilmicin	4 127	19 (4)	0 (0) - 51 (1)
Amikacin	4 227	2 (2)	0 (0) - 7 (0)
Co-trimoxazole	4292	36 (1)	11(0) - 61 (0)

* rezultati centara s malim brojem izolata (<30) nisu uzeti u obzir
results from the centers with small number of isolates (<30) were not taken into consideration

Enterobacter spp., *Serratia* spp., *Citrobacter* spp.
(1.10. - 31.12. 2013.) - osjetljivost na antibiotike u RH
- sensitivity to antibiotics in Croatia



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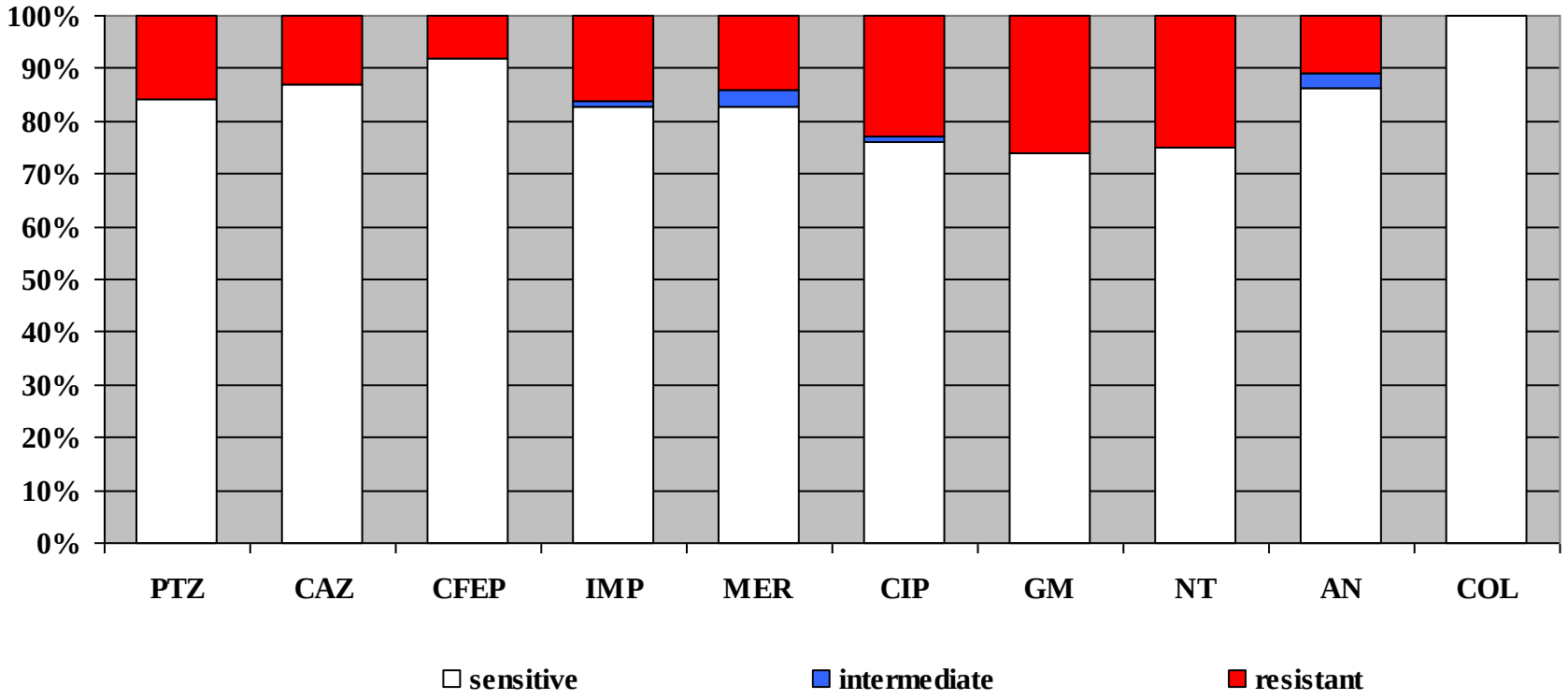
Enterobacter spp., *Serratia* spp., *Citrobacter* spp.

- rezistencija na antibiotike u razdoblju od 1.10.- 31.12. 2013.
zbirni prikaz izolata iz 40 centra u RH
- antibiotic resistance for the period 1.10. - 31.12. 2013.
summary results for the isolates from 40 centers in Croatia

ANTIBIOTIK ANTIBIOTIC	Broj izolata No. of isolates	% rezistentnih (% intermedijarnih) izolata % of resistant (% of intermediate) isolates	Raspon lokalnih rezultata* Range of local results*
Ampicillin	3 244	99 (0)	0 (0) - 100 (0)
Amoxicillin + clav. acid	3 255	88 (0)	58 (0) - 100 (0)
Piperacillin + tazobactam	3 178	15 (3)	0 (2) - 46 (0)
Cephalexin	2 762	90 (0)	57 (0) - 100 (0)
Ceftazidime	3 235	21 (1)	10 (0) - 50 (0)
Ceftriaxone	3 236	22 (1)	10 (0) - 35 (1)
Cefepime	3 184	9 (2)	0 (2) - 37 (0)
Ceftibuten	2 774	25 (0)	6 (0) - 80 (0)
Cefixime	2 700	28 (0)	13 (0) - 62 (0)
Ertapenem	3141	2 (1)	0 (0) - 11 (0)
Imipenem	3 204	1 (0)	0 (0) - 3 (0)
Meropenem	3 203	1 (0)	0 (0) - 2 (1)
Ciprofloxacin	3 242	11 (1)	3(0) - 23 (0)
Norfloxacin	2 738	10 (1)	3 (0) - 50 (0)
Gentamicin	3 244	14 (1)	4 (0) - 28 (7)
Netilmicin	3 058	11 (1)	0 (0) - 23 (0)
Amikacin	2 802	2 (1)	0 (0) - 17 (2)
Co-trimoxazole	2 867	17 (0)	7 (0) - 30 (0)

* rezultati centara s malim brojem izolata (<30) nisu uzeti u obzir
results from the centers with small number of isolates (<30) were not taken into consideration

Pseudomonas aeruginosa (1.10. - 31.12. 2013.) - osjetljivost na antibiotike u RH - sensitivity to antibiotics in Croatia



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Pseudomonas aeruginosa

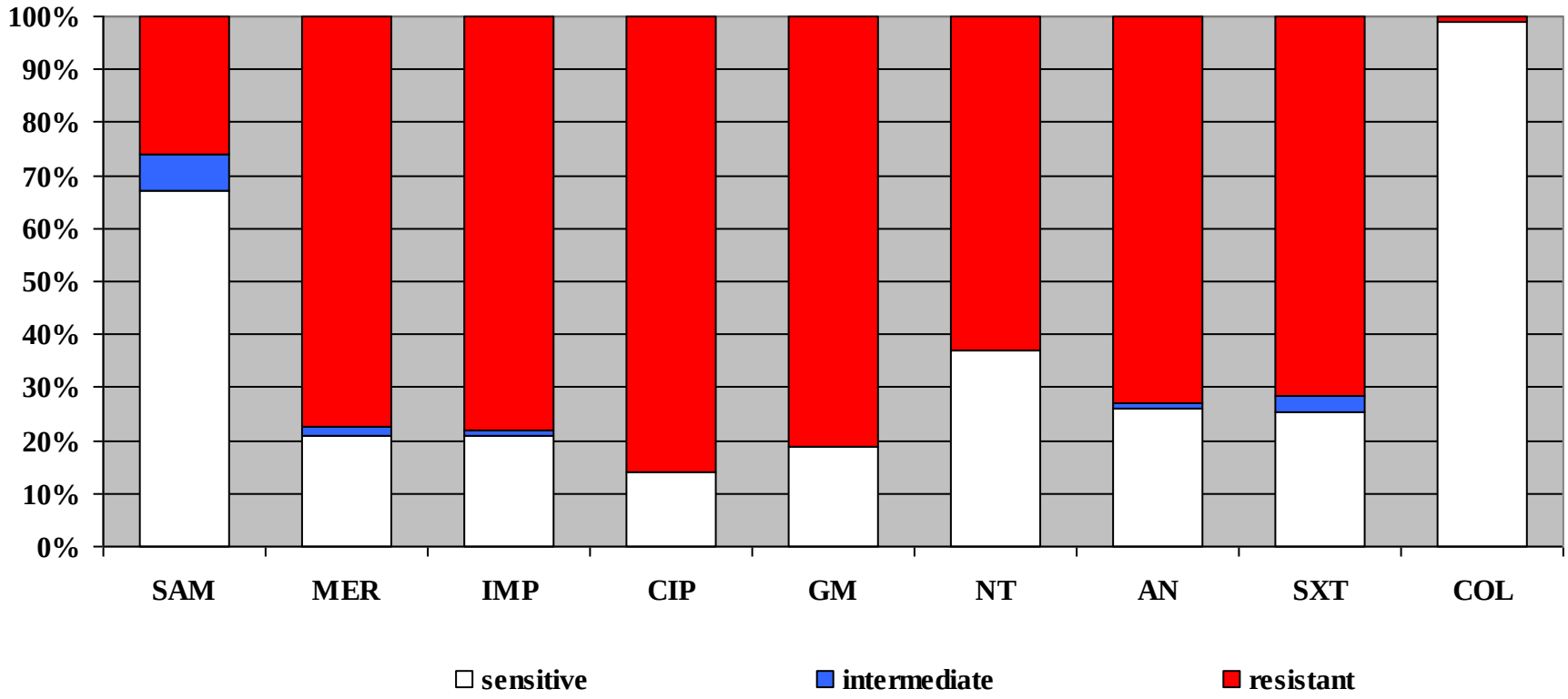
- rezistencija na antibiotike u razdoblju od 1.10.- 31.12. 2013.
zbirni prikaz izolata iz 40 centra u RH
- antibiotic resistance for the period 1.10. - 31.12. 2013.
summary results for the isolates from 40 centers in Croatia

ANTIBIOTIK ANTIBIOTIC	Broj izolata No. of isolates	% rezistentnih (% intermedijarnih) izolata % of resistant (% of intermediate) isolates	Raspon lokalnih rezultata* Range of local results*
Piperacilin + tazobaktam	4 465	16 (0)	0 (0) - 52 (0)
Ceftazidim	4 458	13 (0)	3 (0) - 63 (0)
Cefepim	4 461	8 (0)	0 (0) - 52 (0)
Imipenem	4 456	16 (1)	0(0) - 54 (2)
Meropenem	4 458	14 (3)	0 (0) - 56 (2)
Ciprofloxacin	4 463	23 (1)	6 (3) - 48 (2)
Gentamicin	4 468	26 (0)	7 (0) - 50 (0)
Netilmicin	4 242	25 (0)	0 (0) - 54 (0)
Amikacin	3 870	11 (3)	0 (0) - 36 (0)
Colistin	944	0 (0)	0 (0) - 0(0)

* rezultati centara s malim brojem izolata (<30) nisu uzeti u obzir
results from the centers with small number of isolates (<30) were not taken into consideration

n

Acinetobacter baumannii (1.10. - 31.12. 2013.) - osjetljivost na antibiotike u RH - sensitivity to antibiotics in Croatia



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Acinetobacter baumannii

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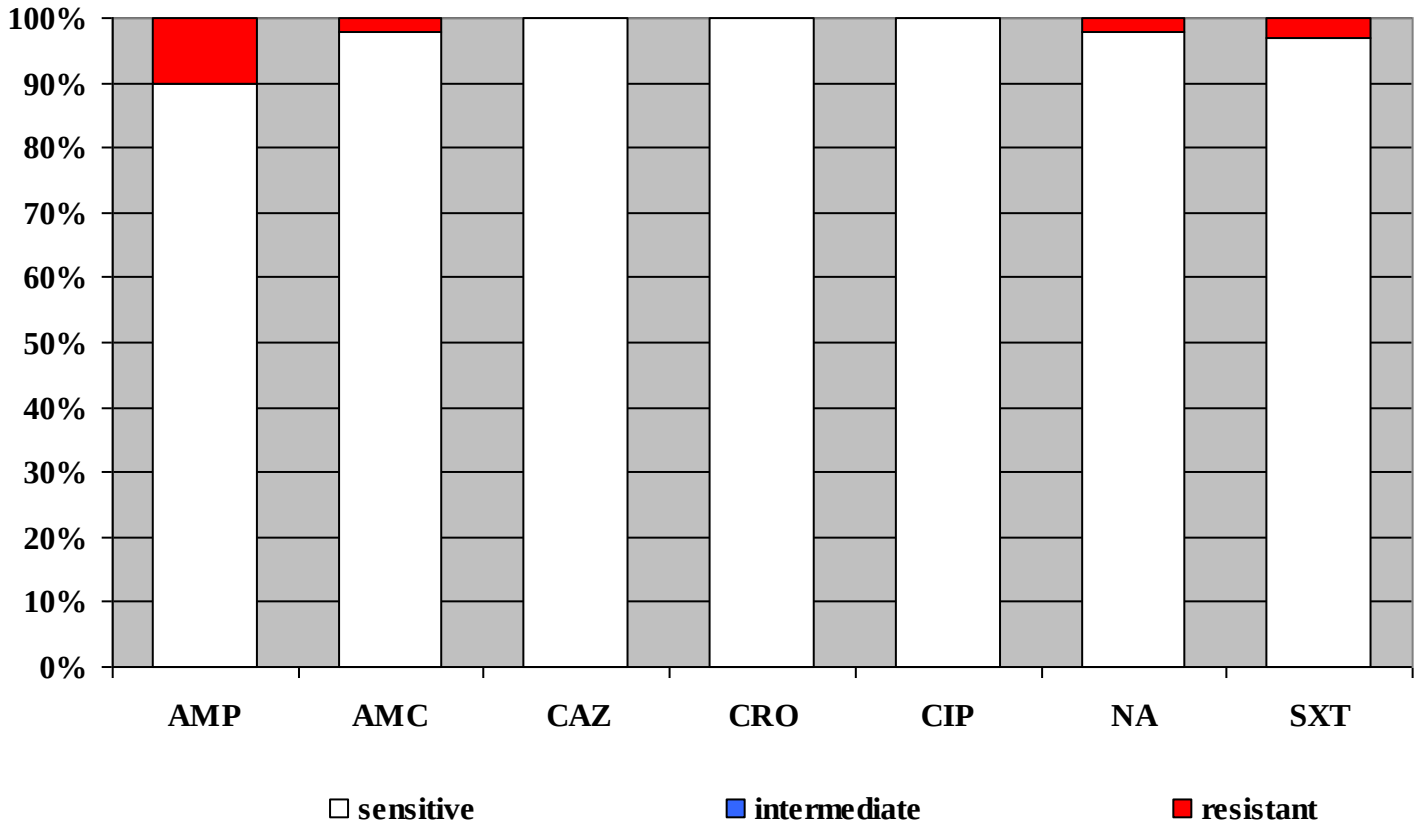
zbirni prikaz izolata iz 40 centra u RH
- antibiotic resistance for the period 1.10. - 31.12. 2013.
summary results for the isolates from 40 centers in Croatia

ANTIBIOTIK ANTIBIOTIC	Broj izolata No. of isolates	% rezistentnih (% intermedijarnih) izolata % of resistant (% of intermediate) isolates	Raspon lokalnih rezultata* Range of local results*
Ampicillin + sulbactam	1 364	26 (7)	1 (0) - 64 (10)
Meropenem	1 411	78 (2)	72 (0) - 94 (0)
Imipenem	1 411	78 (1)	70 (0) - 94 (0)
Ciprofloxacin	1 413	86 (0)	58 (0) - 99 (0)
Gentamicin	1 412	81 (0)	44 (0) - 95 (0)
Netilmicin	1 322	69 (0)	8 (0) - 95 (0)
Amikacin	1 326	73 (1)	44 (0) - 95 (0)
Co-trimaxazole	1 324	71 (3)	40 (2) - 95 (0)
Colistin	1 045	1 (0)	0 (0) - 33 (0)

* rezultati centara s malim brojem izolata (<30) nisu uzeti u obzir
results from the centers with small number of isolates (<30) were not taken into consideration

Salmonella spp. (1.01. - 31.12. 2013.) - osjetljivost na antibiotike u RH - sensitivity to antibiotics in Croatia



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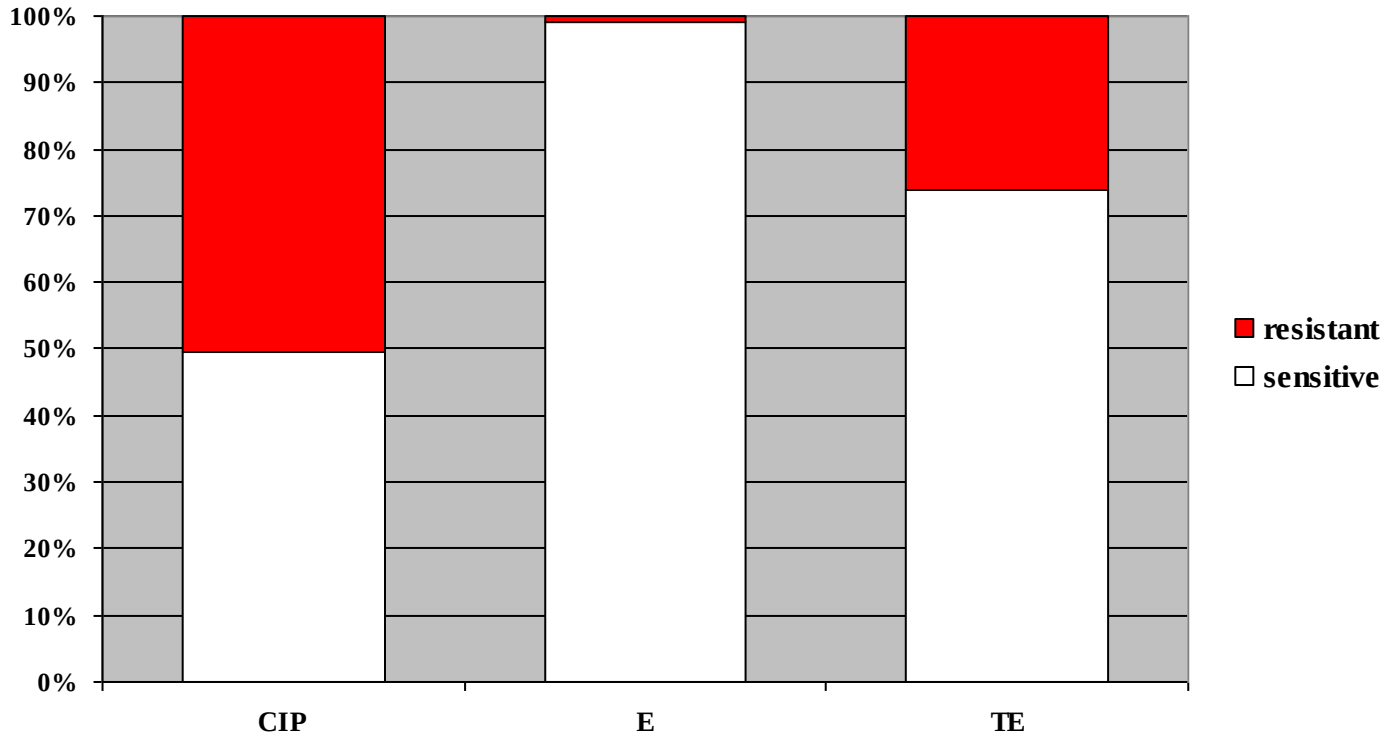
Salmonella spp.

- rezistencija na antibiotike u razdoblju od 01.01.- 31.12. 2013.
zbirni prikaz izolata iz 40 centra u RH
- antibiotic resistance for the period 01.01. - 31.12. 2013.
summary results for the isolates from 40 centers in Croatia

ANTIBIOTIK ANTIBIOTIC	Broj izolata No. of isolates	% rezistentnih (% intermedijarnih) izolata % of resistant (% of intermediate) isolates	Raspon lokalnih rezultata* Range of local results*
Ampicillin	2 158	10 (0)	0 (0) - 38 (0)
Amoxicillin + clav. acid	2 158	2 (0)	0 (0) - 13 (0)
Ceftazidim	2 158	0 (0)	0 (0) - 2 (0)
Ceftriaxone	2 158	0 (0)	9 (0) - 2 (0)
Ciprofloxacin	2 157	0 (0)	0(0) - 3 (0)
Nalidixic acid	2 103	2 (0)	0 (0) - 7 (0)
Co-trimoxazole	2 158	3 (0)	0 (0) - 10 (0)

* rezultati centara s malim brojem izolata (<30) nisu uzeti u obzir
results from the centers with small number of isolates (<30) were not taken into consideration

Campylobacter jejuni (1.01. - 31.12. 2013.) - osjetljivost na antibiotike u RH - sensitivity to antibiotics in Croatia



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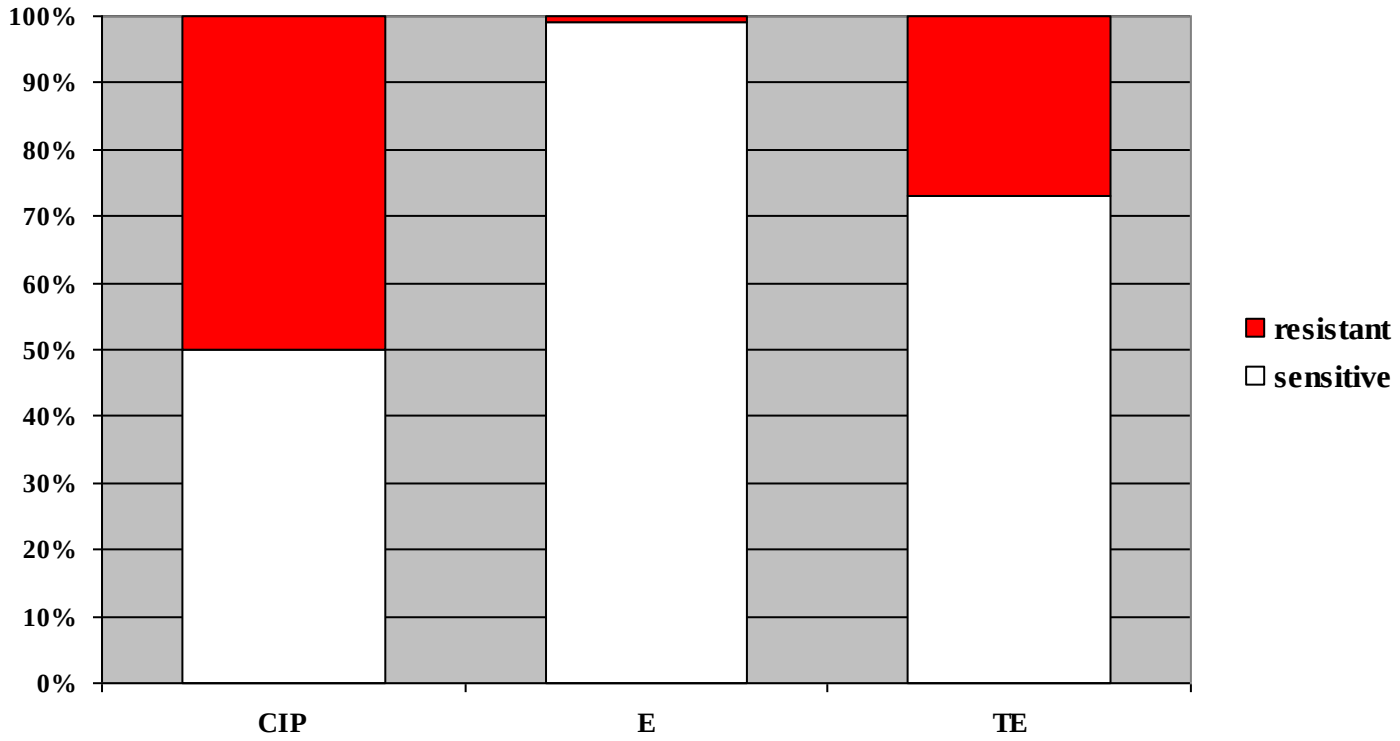
Campylobacter jejuni

- rezistencija na antibiotike u razdoblju od 01.01.- 31.12. 2013.
zbirni prikaz izolata iz 40 centra u RH
- antibiotic resistance for the period 01.01. - 31.12. 2013.
summary results for the isolates from 40 centers in Croatia

ANTIBIOTIK ANTIBIOTIC	Broj izolata No. of isolates	% rezistentnih (% intermedijarnih) izolata % of resistant (% of intermediate) isolates	Raspon lokalnih rezultata* Range of local results*
Ciprofloxacin	2 146	50 (0)	0 (0) - 67 (0)
Erythromicin	2 145	1 (0)	0 (0) - 34 (0)
Tetracycline	1 549	26 (0)	12 (0) - 45 (0)

* rezultati centara s malim brojem izolata (<30) nisu uzeti u obzir
results from the centers with small number of isolates (<30) were not taken into consideration

Campylobacter coli (1.01. - 31.12. 2013.) - osjetljivost na antibiotike u RH - sensitivity to antibiotics in Croatia



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Campylobacter coli

- rezistencija na antibiotike u razdoblju od 01.01.- 31.12. 2013.
zbirni prikaz izolata iz 40 centra u RH
- antibiotic resistance for the period 01.01. - 31.12. 2013.
summary results for the isolates from 40 centers in Croatia

ANTIBIOTIK **ANTIBIOTIC**	**Broj izolata** **No. of isolates**	**% rezistentnih** **(% intermedijarnih) izolata** **% of resistant** **(% of intermediate) isolates**	**Raspon lokalnih rezultata*** **Range of local results***
Ciprofloxacin	698	50 (0)	42 (0) – 56 (0)
Erythromicin	698	1 (0)	0 (0) – 3 (0)
Tetracycline	494	27 (0)	25 (0) – 31 (0)

* rezultati centara s malim brojem izolata (<30) nisu uzeti u obzir
results from the centers with small number of isolates (<30) were not taken into consideration

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***Shigella* spp.** - rezistencija na antibiotike u RH / antibiotic resistance in Croatia, 01.01 - 31.12.2013.

	AMP			AMC			CAZ			CRO			CIP			SXT		
***Shigella* spp.**	No	I %	**R %**	No	I %	**R %**	No	I %	**R %**	No	I %	**R %**	No	I %	**R %**	No	I %	**R %**
Shigella* sonnei	24	0	**0**	24	0	**0**	24	0	**0**	24	0	**0**	24	0	**9**	24	0	**92**
Shigella* flexneri	3	0	**33**	3	0	**0**	3	0	**0**	3	0	**0**	3	0	**0**	3	0	**67**
UKUPNO* ***TOTAL***	27	0	**4**	27	0	**0**	27	0	**0**	27	0	**0**	27	0	**13**	27	0	**89**

* podatak o postotku rezistencije nepouzdan zbog premalo izolata
resistance rate data unreliable due to small number of isolate

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Anaerobne bakterije - rezistencija na antibiotike u RH / antibiotic resistance in Croatia, 01.01 - 31.12.2013.

Anaerobes

	P			AMC			PTZ			ERT			MTZ			CC		
	No	I %	**R %**	No	I %	**R %**	No	I %	**R %**	No	I %	**R %**	No	I %	**R %**	No	I %	**R %**
Gram pozitivni anaerobi osim C.difficile	295	1	**12**	317	1	**2**	294	2	**2**	191	0	**1**	312	0	**50**	311	0	**15**
Gram negativni anaerobi	373	0	**85**	396	1	**10**	377	4	**6**	256	0	**1**	383	0	**12**	390	0	**27**
UKUPNO ***TOTAL***	668	1	**53**	713	1	**6**	671	3	**4**	447	0	**1**	695	0	**29**	701	0	**21**

POGLAVLJE/CHAPTER 2.

OSJETLJIVOST *M. TUBERCULOSIS* U HRVATSKOJ U 2013. GODINI
SENSITIVITY OF M. TUBERCULOSIS IN CROATIA, 2013

Prim. Vera Katalinić-Janković, dr. med.
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Odjel za dijagnostiku tuberkuloze
Croatian National Institute of Public Health
Microbiology Service
Mycobacteriology Department

HRVATSKI ZAVOD ZA JAVNO ZDRAVSTVO
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Odjel za tuberkulozu
Microbiology Service
Mycobacteriology Department

Prim. dr. Vera Katalinić-Janković

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Mikobakterije izolirane u Hrvatskoj u 2013. godini

Incidencija tuberkuloze u 2013. godini je zadržala isti trend (13/100 000), a mreža TBC laboratorija je ostala nepromijenjena (14 laboratorija). Ukupno je pregledano 39.608 kliničkih uzoraka na tuberkulozu što je za 10% manje nego u 2012. godini. U 4,4% uzoraka kultivacijom su otkrivene mikobakterije, a raspon pozitivnih kultura među laboratorijima se kretao od 0,4 do 15,4% pozitivnih uzoraka. Ukupno je izolirano 2.153 sojeva mikobakterija što je na razini broja izolata u 2012. godini. Međutim, iako je *M. tuberculosis* i dalje dominantna mikobakterija s 1.748 (81,2%) izolata, udio netuberkuloznih mikobakterija (NTM) je porastao na 18,8%. Tijekom 2013. godine iz humanih kliničkih materijala nije izoliran *M. bovis*, a zabilježen je samo 1 *M. bovis* – BCG soj. Osobe s izolatima NTM se bilježe od 1982. godine, a kod višekratnih izolacija se utvrđuju mikrobiološki kriteriji za mikobakterioze i popunjava obrazac za NTM. U 2013. godini je otkriveno 28 (0,65/100.000) bolesnika s zadovoljenim mikrobiološkim kriterijima za dijagnozu mikobakterioze. Kod 8 bolesnika uzročnik mikobakterioze je bio *M. xenopi, M. abscessus* kod 6 bolesnika, *M. kansasii* kod 5, *M. avium* i *M. intracellulare* kod 4 bolesnika. Registriran je i jedan bolesnik gdje je izolirani uzročnik bio *M. intermedium.* U odnosu na prethodnih 10 godina zabilježen je zamjetan porast broja mikobakterioza gdje su uzročnici bili *M. kansasii*, odnosno *M. abscessus*. *M. gordonae* kao saprofitna mikobakterija je identificiran u 29,3% NTM izolata. Najčešće se radilo o kontaminaciji uzoraka, slučajnim nalazima i opetovanim pseudoinfekcijama u više zdravstvenih ustanova. Među uvjetno patogenim NTM u Hrvatskoj i dalje prevladavaju *M. xenopi* s 23,1% i *M. fortuitum* s 11,2% izolata. Iako je *M. kansasii* otkriven u samo 4,0% izolata, gotovo svi izolati ove vrste su bili klinički značajni.

Izrazito povoljan trend broja rezistentnih sojeva *M. tuberculosis*, a time i bolesnika s rezistentnom tuberkulozom je nastavljen. Od 1.748 izoliranih sojeva *M. tuberculosis* samo je 91 (5,2%) bilo rezistentno na prvu liniju antituberkulotika. Među rezistentnim sojevima 62,6% je bilo monorezistentno, dok je 37,4% izolata bilo rezistentno na 2 i više antituberkulotika iz prve linije. Radi se o izolatima *M. tuberculosis* kod dugogodišnjih kroničnih bolesnika s rezistentnom tuberkulozom. Monorezistencija na izoniazid (H) je utvrđena kod 48,3% izoliranih sojeva, a monorezistencija na streptomicin (S) kod 13,2%. Ovi nalazi monorezistencije ukazuju da je u Hrvatskoj rezistencija na H i dalje najznačajniji prekursor multirezistencije i zahtjeva ozbiljan pristup u liječenju ovih bolesnika. Rezistencija na antituberkulotike kod *M. tuberculosis* nastaje spontanim mutacijama u specifičnim regijama određenih gena. Oko 96% sojeva rezistentnih na R imaju mutaciju u regiji gena *rpoB* dugačkoj 81 pb, a rezistencija na H povezana je s brojnim mutacijama koje pogađaju jedan ili više gena od kojih su najznačajniji geni *katG* i *inhA*. Na Odjelu za dijagnostiku tuberkuloze za određivanje mutacija u genima *rpoB*, *katG* i *inhA* koriste se komercijalni test Genotype MTBDR*plus* (Hain Lifescience) i *in house* metoda višestrukog PCR uz korištenje specifičnih

početnica koje su načinjene tako da otkrivaju postojanje mutacija u genima *katG* (Ser315Thr) i *inhA* (*inhA*$^{C\text{-}15T}$). Navedenim metodama bilo je moguće odrediti molekularnu osnovu rezistencije na R kod svih sojeva izoliranih u bolesnika s multirezistentnom tuberkulozom u 2013. godini. U istih bolesnika molekularna osnova rezistencije na H određena je samo za jedan soj - dokazana je mutacija u genu *katG*. Za preostala 3 multirezistentna soja dostupnim metodama nije bilo moguće odrediti molekularnu osnovu rezistencije na H. U 2013. godini izolirana su i dva polirezistentna soja čiji je profil rezistencije uključivao rezistenciju na H uzrokovanu mutacijom u genu *katG*. Među sojevima koji su bili monorezistentni na H prevladavala je mutacija u genu *inhA* (60%). Kako za ukupno 4 (25%) soja rezistentna na H (neovisno o profilu rezistencije) nije bilo moguće odrediti molekularnu osnovu rezistencije, još uvijek nije moguće u potpunosti zamijeniti fenotipsko ispitivanje osjetljivosti na ATL molekularnim testovima.

U 2013. godini metodom MIRU-VNTR (engl. Mycobacterial Interspersed Repetitive Units - Variable Number of Tandem Repeats) uz korištenje 15 MIRU lokusa, genotipizirana su 353 klinička izolata *M. tuberculosis*, a od početka prospektivne populacijske studije genotipizacije svih novoizoliranih sojeva (2004. godine) više od 6.100 sojeva. Genotipizacijom sojeva *M. tuberculosis* omogućeno je razdvajanje endogene reaktivacije prethodne infekcije od superinfekcije, praćenje prijenosa infekcije na nacionalnoj ili lokalnoj razini (posebice rezistentnih sojeva) te otkrivanje moguće intralaboratorijske kontaminacije. Rezultati genotipizacije korištenjem ove metode su numerički pa ih je moguće jednostavno uspoređivati unutar laboratorija te s referentnim sojevima iz mrežnih baza podataka kao što je baza podataka MIRU-VNTRplus (www.miru-vntrplus.org).

Mycobacteria isolated in Croatia in 2013

TB incidence hit an all-time low in Croatia in 2013 with a rate of 13/100,000 inhabitants. The number of TB laboratories did not change, though, and the diagnostic was divided between 14 labs on three levels. To analyze data on isolated strains, a questionnaire on the work of TB laboratories in 2013 was used.

A total of 39,608 clinical samples were analyzed for tuberculosis. In 4.4% of samples, cultivation detected mycobacteria and the range of positivity of cultures in different laboratories was from 0.4 to 15.4%. Similar to 2012, a total of 2.153 mycobacterial strains were isolated. *M. tuberculosis* remained the predominant mycobacterium with 1,748 (81.2%) isolates, though on a lower scale than the previous year. The number of nontuberculous mycobacteria (NTM) increased from 14.0% in 2012 to 18.8% in 2013. No *M. bovis* strains and only one *M. bovis* BCG strain were isolated in 2013 (Table 1).

Mycobacterioses are not reported to the Epidemiology Service in Croatia. Lab data on cases with multiple NTM isolates have, still, been systematically documented since 1982. Though the number of mycobacterioses is relatively small, the absolute number of cases in the monitored period is continually on the rise or stabile. In 2013, a total of 28 (0.65/100,000) cases fulfilling the microbiological criteria for mycobacteriosis were documented. The cause of mycobacteriosis in 8 patients was *M. xenopi*, in 6 *M. abscessus*, in 5 *M. kansasii*, and in 4 each *M. avium* and *M. intracellulare*, respectively. One patient with isolated *M. intermedium* was recorded. Comparing to last 10 years, there has been an increase in number of mycobacterioses caused by either *M. kansasii* or *M. abscessus*. *M. gordonae*, a saprophytic mycobacterium, was identified in 29.3% NTM isolates. In most cases, the isolation was the result of specimen contamination, accidental finding and repeated pseudoinfections in several health care facilities. Among opportunistic pathogenic NTM in Croatia still prevail *M. xenopi* (23.1%) and *M. fortuitum* (11.2% isolates). Although *M. kansasii* was detected in only 4.0% isolates, almost all isolates of this species were clinically significant.

The number of resistant *M. tuberculosis* strains and, by extension, cases of resistant TB has not demonstrated any significant increase. Of the 1,748 isolated *M. tuberculosis* strains, only 91 (5.2%) were resistant to the first line antituberculotics (Table 3). Among drug resistant strains 62.6% were monoresistant, while over 37.4% of *M. tuberculosis* isolates were resistant to 2 or more first-line antituberculotics. Monoresistance to isoniazid (H) was established in 48.3% of isolated cases, and monoresistance to streptomycin (S) in 13.2% isolated cases (Table 4). These findings suggest that the mono-drug resistance to H is still possible precursor of multiresistance and requires a serious approach to the treatment of patients with monoresistant tuberculosis. Resistance to antituberculotics in *M. tuberculosis* is caused by spontaneous mutation in specific regions of certain genes. Some 96% of strains resistant to R have a mutation in the 81-pb-long region of the *rpoB* gene, while resistance to H is related to the numerous mutations affecting one or more genes, most significant being *katG* and *inhA*. At the TB Diagnostics Department of the Croatian National Institute of Public Health, to determine resistance conferring mutations in the *rpoB*, *katG* and *inhA* genes, commercial Genotype MTBDR*plus* (Hain Lifescience) tests and an in-house multiplex PCR method are used, with specific primers designed for detecting mutation in genes *katG* (Ser315Thr) and *inhA* (*inhA*$^{C\text{-}15T}$). The molecular basis of the resistance to R using said methods was determinable in all 4 patients with multiresistant TB in 2013, while the resistance to H could be determined in only one strain with the mutation in *katG* gene, the mutation that often precedes further acquiring of resistance, especially multiresistance. In 2013 there were 10 strains with monoresistance to H isolated; in 75% of these strains, molecular basis of resistance to H was determined. Both polyresistant strains, whose resistance profile included the resistance to H developed the mutation in *katG* gene (Table 5). Still, as for 4 (25%) of

strains the molecular base of resistance to H could not be determined, phenotypic test of sensitivity to ATL can still not be substituted by molecular tests.

In 2013, using 15 loci MIRU-VNTR (Mycobacterial Interspersed Repetitive Units - Variable Number of Tandem Repeats) method, a total of 353 clinical *M. tuberculosis* isolates were genotyped, and since the beginning of the prospective population study of genotyping of all newly isolated *M. tuberculosis* strains (in 2004), more than 6,100 strains. Genotyping of *M. tuberculosis* strains enables differentiating between endogenous activation of previous infection and superinfection, following infection transmission on national or local level (especially resistant strains) and confirming or refuting intra-laboratory contamination. When using this method, genotyping results are numerical, thus enabling simple comparison of data both within the laboratory registry as well comparison with the referent strains in web databases such as MIRU-VNTRplus database (www.miru-vntrplus.org).

Tablica-*Table* 1.
Mikobakterije izolirane u Hrvatskoj, 2003. –2013.
Mycobacteria strains isolated in Croatia, 2003-2013

Godina **Year**	**Ukupno mikobakterija** **Total**	**M. tuberculosis**		***M. bovis***		**Netuberkulozne mikobakterije** *Nontuberculous mycobacteria*	
		Broj ***No***	**%**	***M. bovis***	**BCG -soj**	**Broj** ***No***	**%**
2003	4760	4516	94,8	-	1	243	5,1
2004	4170	3958	94,9	1	3	208	5,0
2005	4114	3904	94,9	-	-	210	5,1
2006	3959	3717	93,9	-	2	240	6,1
2007	3217	2920	90,8	1	4	292	9,1
2008	3665	3299	90,0	-	1	365	9,9
2009	3197	2763	86,4	-	-	434	13,6
2010	2712	2283	84,2	-	1	429	15,8
2011	2351	2000	85,0	-	4	347	14,8
2012	2108	1807	85,7	1	6	294	14,0
2013	2153	1748	81,2	-	1	402	18,8

Tablica-*Table* 2.
Netuberkulozne mikobakterije (NTM) izolirane u Hrvatskoj u 2013. godini
Nontuberculous mycobacteria (NTM) isolated in Croatia in 2013

	Vrsta	**Broj**	**%**
UVJETNO PATOGENE MIKOBAKTERIJE			
sporog rasta	*M. avium*	15	3,7
	M. intracellulare	25	6,2
	M. kansasii	16	4,0
	M. xenopi	93	23,1
	M. intermedium	4	1,0
	M. scrofulaceum	1	0,3
brzog rasta	*M. fortuitum*	45	11,2
	M. chelonae	31	7,7
	M. abscessus	25	6,2
	M. mucogenicum	4	1,0
	M. celatum	2	0,5
SAPROFITNE MIKOBAKTERIJE			
sporog rasta	*M. gordonae*	118	29,3
	M. terrae	11	2,7
	M. nonchromogenicum	3	0,7
	M. thermoresistibile	2	0,5
brzog rasta	*M. flavescens*	1	0,3
	M. vaccae	3	0,7
	M. aurum	1	0,3
	M. phlei	1	0,3
	Mycobacterium sp.	1	0,3
Ukupno		**402**	**100**

Tablica-*Table* 3.
Osjetljivost sojeva *M. tuberculosis* na antituberkulotike u Hrvatskoj, 2013. g.
Drug Susceptibility Testing of M. tuberculosis strains in Croatia, 2013

Ustanova Institution	**M. tuberculosis** *strains*	***Osjetljivi*** Sensitive	***Rezistentni*** Resistant
ZJZ Čakovec	30	30	-
SB Klenovnik	617	569	48
OB Nova Gradiška	48	48	-
ZJZ Osijek	93	89	4
ZJZ Pula	52	52	-
ZJZ Rijeka	119	116	3
ZJZ Sl.Brod	43	43	-
KBC Split	70	66	4
ZJZ Split	5	5	-
ZJZ Šibenik	21	21	-
ZJZ Virovitica	4	4	-
ZJZ Zadar	57	47	10
KBC Zagreb	162	160	2
HZJZ	427	407	20
Ukupno	**1748**	**1657**	**91**

Tablica-*Table* 4.
Rezistentni sojevi M. tuberculosis u Hrvatskoj, 2013. godina
Drug resistant M. tuberculosis strains isolated in Croatia in 2013

1 ATL	**Broj sojeva (No.)**
S (streptomicin)	12 (13,2%)
H (izoniazid)	44 (48,3%)
R (rifampicin)	-
E (etambutol)	1 (1,1%)
Z (pirazinamid)	-
	57 (62,6%)
2 ATL	
S,H	4 (4,4%)
	4 (4,4%)
3 ATL	
H,R,S	-
H,R,E	-
H,R,Z	-
S,R,Z	-
S,H,Z	-
4 i 5 ATL	
S,H,R,E	-
S,R,R,Z	2 (2,2%)
S,H,R,E,Z	28 (30,8%)
	30 (33,0%)
Ukupno - *Total*	**91** (100,0%)

Legenda - *Key*: ATL – antituberkulozni lijekovi
antituberculotic drugs

Tablica-*Table* **5.**
Mutacije odgovorne za rezistenciju na rifampicin i izoniazid u 2013. godini
Mutations responsible for rifampicin and isoniazid resistance in 2013

	Br. bolesnika *- No of patients*	***katG***	***%***	***inhA***	***%***	***WT***	***%***	***rpoB***	***%***
MDR	4	1	25,0	0	/	3	75,0	4	100
Monorezistentni- *Monoresistant*	10	3	30,0	6	60,0	1	10,0	/	/
Polirezistenti- *Polyresistant*	2	2	100	0	/	0	/	/	/
Ukupno - *Total*	16	6	37,5	6	37,5	4	25,0		

POGLAVLJE/CHAPTER 3.

PRAĆENJE REZISTENCIJE NA ANTIBIOTIKE U INVAZIVNIH IZOLATA
ANTIBIOTIC RESISTANCE SURVEILLANCE IN INVASIVE ISOLATES

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Važnost praćenja rezistencije u invazivnih izolata

Sustavno praćenje rezistencije na antibiotike na europskoj razini započelo je 1999.g. u okviru European Antimicrobial Resistance Surveillance System (EARSS) projekta. Za prioritete u praćenju odabrano je u početku šest bakterijskih vrsta *S. aureus, E. faecalis, E. faecium, S. pneumoniae* i *E. coli*, od 2005.g. dodano je praćenje rezistencije u *K. pneumoniae* i *P. Aeruginosa*, a od 2013.g. kao pilot projekt započeto je i praćenje rezistencije u *Acinetobacter* spp. S obzirom na različitu praksu uzimanja uzoraka i interpretaciju nalaza u različitim zemljama odlučeno je da se u praćenju na europskoj razini u obzir uzimaju samo invazivni izolati (iz hemokultura i likvora). Interpretacija nalaza ovih bakterija u hemokulturi i likvoru je u svim laboratorijima jednaka i njihovo kliničko značenje je neupitno. S obzirom na već postojeću mrežu mikrobioloških laboratorija u okviru Odbora za praćenje rezistencije na antibiotike Hrvatska se spremno uključila u EARSS projekt od samog početka, a nakon što je Hrvatska postala članicom Europske unije hrvatski podaci su uključeni u EARS-Net program Europskog centra za prevenciju i kontrolu bolesti (engl. "European Center for Disease Prevention and Control", ECDC). Nedostatak praćenja rezistencije samo u invazivnih izolata je mali broj izolata u nekim centrima što onemogućuje analizu na razini pojedinih centara te činjenica da se prvi izolati s novim mehanizmima rezistencije ne moraju javiti u hemokulturi ili likvoru. Prednost sudjelovanja u europskoj mreži je mogućnost uspoređivanja s drugim zemljama te raspolaganje podacima o rezistenciji među invazivnim izolatima. Masovno praćenje rezistencije opisano u prvom poglavlju ove publikacije i ciljano praćenje invazivnih izolata dobro se nadopunjuju i predstavljaju dobru kombinaciju za praćenje rezistencije u Hrvatskoj na nacionalnoj i lokalnoj razini.

Rezultati praćenja rezistencije u invazivnih izolata

U 2013.g. prikupljen je veći broj izolata negoli prošle godine. Broj laboratorija i broj prikupljenih invazivnih izolata pojedinih vrsta prikazani su u Tablici 1.

Podaci o izolatima šalju se na formularu i obrađuju u Referentnom centru za praćenje rezistencije na antibiotike u Klinici za infektivne bolesti. Sa svrhom retestiranja izolata s rijetkim fenotipom i eventualne daljnje obrade invazivni izolati *S. pneumoniae, E. coli, K. pneumoniae* i *Acinetobacter* spp se šalju u Referentni centar za praćenje rezistencije, a izolati *S. aureus, E. faecalis, E. faecium* i *P. aeruginosa* u Referentni Centar za bolničke infekcije. Tijekom 2013.g. prikupljeno je 119 izolata *S. pneumoniae,* 1066 izolata *E. coli,* 396 izolata *K. pneumoniae,* 533 izolata *S. aureus,* 250 izolata enterokoka (175 *E. faecalis* i 75 *E. faecium* izolata), 256 izolata *P. aeruginosa* , te 114 izolata *Acinetobacter* spp. (Tablica 1). U 2013.g. podaci o invazivnim izolatima su stigli iz 21 centra.

U odnosu na prethodnu godinu uočen je lagani porast rezistencije za većinu antibiotika (Tablica 2).

Primjetan je trend porasta stope penicilin neosjetljivih invazivnih izolata penumokoka (27%) koja dostiže stopu neosjetljivosti izolata koji koloniziraju nazofarinks (31%). Ipak, samo 4% invazivnih izolata nije dostupno parenteralnoj terapiji penicilinom ukoliko infekcija ne zahvaća središnji živčani sustav te se parenteralni penicilin još uvijek može smatrati lijekom izbora za liječenje pneumokoknih pneumonija. Rezistencija na makrolide (34%) je također u porastu.

Udio MRSA izolata (24%) nažalost ne pokazuje daljni pad, već je u laganom porastu, ali se i dalje drži vrijednosti ispod 30% kao što je to po prvi puta registrirano 2010. godine. Taj trend odgovara sniženju stope MRSA među svim stafilokokima bez obzira na vrstu uzorka (12% u 2013.g.).

Udio enterokoka rezistentnih na glikopeptide je u porastu (7%) u odnosu na prijašnje godine, a stopa visoke rezistencije na aminoglikozide je i dalje visoka.

Rezistencija *E. coli* na fluorokinolone (21%) je u porastu u odnosu na prošlogodišnju stopu (17%). Udio sojeva *E. coli* koji proizvode beta-laktamaze proširenog spektra (engl. „extended spectrum beta-lactamases“, ESBL) je u laganom porastu dok je udio *K. pneumoniae* izolata rezistentnih na 3. generaciju cefalosporina podjednak prošlogodišnjim vrijednostima. Iako je prvi invazivni izolat *K. pneumoniae* rezistentan na karbapeneme opisan 2009.g. po prvi puta u 2013.g. neosjetljivost invazivnih *K. pneumoniae* na karbapeneme se ispoljila kao 1%.

Karbapenem rezistentni *P. aeruginosa* je još uvijek veliki problem u Hrvatskoj. Lagani pad u rezistenciji na većinu antibiotika uočen prošle godine nije se, nažalost, potvrdio u ovogodišnjim rezultatima.

U okviru masivnog praćenja rezistencije u izolata iz svih uzoraka uočen je već 2009.g. nagli porast rezistencije *Acinetobacter* spp. na karbapeneme. U 2013.g. po prvi puta se rezistencija pratila u invazivnih izolata acinetobaktera i očekivano pokazala izuzetno visoki postotak rezistencije na karbapeneme (91%).

Demografski podaci za pacijente i porijeklo uzoraka prikazani su u tablicama 3 i 4.

Zastupljenost rezistentnih izolata u pojedinim centrima prikazana je na slikama 1- 8.

Impact of antibiotic resistance surveillance in invasive isolates

Systematic antibiotic resistance surveillance at the European level started with the European Antimicrobial Resistance Surveillance System (EARSS) project in 1999. At the beginning six bacterial species were selected as a priority for resistance surveillance, namely *S. aureus, E. faecalis, E. faecium, S. pneumoniae* and *E. coli*, in 2005 *K. pneumoniae* and *P. aeruginosa* were added and in 2013 *Acinetobacter* spp isolates were included as a pilot project. Considering that there is a wide variation in sampling and interpretation of results among different countries it was decided that only invasive isolates (from bloodcultures and cerebrospinal fluid, CSF) will be included in the European surveillance. Interpretation of bacterial growth in blood and CSF is unique for the species tested in all laboratories and the clinical significance of these findings is not in question. Thanks to the already existing network of microbiology laboratories within the Croatian Committee for Antibiotic Resistance Surveillance, Croatia readily joined EARSS at the very beginning of the project and when Croatia joined the European Union, Croatian data got included into EARS-Net program of the European Center for Disease Prevention and Control (ECDC). The limitation of antibiotic resistance surveillance in invasive isolates only, is that some centres may have too few isolates to enable analysis at the local level and first isolates with novel resistance mechanisms do not necessarily appear in blood or CSF. The advantages of participating in the European surveillance network are the possibility to compare data between countries and obtaining information about resistance in invasive isolates. Therefore mass surveillance as described in chapter 1 of this publication and focused study of resistance in invasive isolates provide a good combination for surveillance of antimicrobial resistance at local and national level in Croatia.

Results of the antibiotic resistance surveillance in invasive isolates

In 2013 a greater number of isolates was collected than the previous year. Number of laboratories reporting and number of invasive isolates collected are shown in Table 1.

Forms with data for each isolate are sent to and analysed at the Reference Centre for Antimicrobial Resistance Surveillance at the University Hospital for Infectious Diseases. With a purpose of retesting and further analysis of isolates with unusual phenotype isolates of *S. pneumoniae*, *E. coli*, *K. pneumoniae* and *Acinetobacter* spp. are sent to the Reference Centre for Antimicrobial Resistance Surveillance while isolates of *S. aureus, E. faecalis, E. faecium* and *P. aeruginosa* are sent to the Reference Centre for Hospital Infections. During 2013 we have collected 119 isolates of *S. pneumoniae*, 1066 isolates of *E. coli*, 396 isolates of *K. pneumoniae*, 533 isolates of *S.aureus*, 250 enterococcal isolates (175 *E. faecalis* and 75 *E. faecium* isolates), 256 isolates of *P.aeruginosa* and 114 isolates of *Acinetobacter* spp. (Table 1). In 2013 data on invasive isolates were collected from 21 centers.

There is a slight increase in resistance for majority of antibiotics as compared with the previous year (Table 2).

There is an increasing trend in non-susceptibility to penicillin in invasive pneumococcal isolates with rate of non-susceptibility (27%) reaching the rate of non-susceptibility in non-invasive isolates (31%). However, only 4% of invasive isolates can not be treated with parenteral penicillin in infections other than central nervous system infections, so penicillin

still remains a drug of choice for treatment of pneumococcal pneumonia. Resistance to macrolides (34%) is increasing as well.

Unfortunately, the proportion of MRSA isolates (24%) has slightly increased, but still remains below 30% which was first recorded in 2010. This decreasing trend in MRSA rates is also observed in surveillance of all staphylococcal isolates regardless of the site of isolation (12% in 2013).

The proportion of glycopeptide resistant enterococci is increasing (7%) and the rate of high level aminoglycoside resistance is still high.

Quinolone resistance in *E. coli* (21%) increased compared to the last year rate (17%). Proportion of *E. coli* isolates producing extended spectrum beta-lactamases (ESBL) slightly increased while number of *K. pneumoniae* isolates resistant to 3rd generation cephalosporins still remained the same. Although carbapenem resistant *K. pneumoniae* was first reported in 2009, carbapenem resistance in invasive *K. pneumoniae* reached 1% for the first time in 2013.

Carbapenem resistance in *P. aeruginosa* still remaines a big problem in Croatia. Slight decrease in resistance recorded last year unfortunately did not continue in 2013.

A sudden increase of carbapenem resistance in *Acinetobacter* spp. was recorded since 2009 among isolates in mass surveillance program. In 2013 data for invasive isolates were collected for the first time and as expected carbapenem resistance in invasive isolates (91%) is very high as well.

Demographic patient data and sample origin data are shown in Tables 3 and 4.

Proportion of resistant strains by laboratory centres is shown in Figures 1- 8.

Tablica-*Table 1.*

Broj laboratorija i izolata prijavljenih u razdoblju od 2001.-2013.

Number of laboratories and number of isolates reported for the period 2001-2013

Godina	*S. pneumoniae*		*S. aureus*		*E.coli*		*Enterococcus* spp.		*K.pneumoniae*		*P. aeuroginosa*		*Acinetobacter spp.*	
	Lab	Izolati / Isolates	Lab	Izolati/ Isolates	Lab	Izolati/ Isolates	Lab	Izolati/ Isolates	Lab	Izolati/ Isolates	Lab	Izolati/ Isolates	Lab	Izolati/ Isolate
2001	10	20	14	149	13	182	7	33	0	0	0	0		
2002	14	90	14	279	15	490	13	96	0	0	0	0		
2003	12	88	14	360	16	570	11	101	0	0	0	0		
2004	12	103	13	392	14	535	11	115	0	0	0	0		
2005	15	129	17	354	16	638	11	120	14	112	10	72		
2006	14	116	17	391	17	780	16	178	15	205	15	170		
2007	15	136	15	375	17	852	13	174	17	279	16	189		
2008	13	100	18	474	17	915	16	232	17	333	14	221		
2009	14	100	14	463	16	911	20	223	16	318	15	212		
2010	11	103	15	363	16	897	12	176	16	286	15	217		
2011	16	127	14	451	16	1007	15	244	14	314	15	265		
2012	11	98	17	412	17	921	14	219	15	344	14	204		
2013	16	119	21	533	20	1066	17	250	19	396	19	256	13	114

Tablica-*Table 2.*
Udio izolata smanjene osjetljivosti na antibiotike izražen u postocima
Proportion of antibiotic non-susceptible isolates in percent

PATOGEN / PATHOGEN	ANTIBIOTICI/ Antimicrobial classes	2001 %	2002 %	2003 %	2004 %	2005 %	2006 %	2007 %	2008 %	2010 %	2011 %	2012 %	2013 %
S. pneumoniae	Penicillin R	1	1	1	3	1	1	1	4	7	1	1	4
	Penicillin I+R	15	19	20	17	17	18	18	17	21	18	23	27
	Macrolides I+R	15	23	18	19	17	16	8	14	29	24	28	34
S. aureus	Oxacillin/Met R	32	37	37	38	37	36	38	35	27	27	22	24
E. coli	Aminopenicillins R	51	47	46	45	46	51	51	53	55	55	52	54
	Aminoglycosides R	6	7	7	6	5	6	6	6	6	7	7	7
	Fluoroquinolones R	5	5	7	8	9	15	13	15	17	20	17	21
	3. gen Cef R	2	3	4	3	1	1	3	4	8	7	8	9
	ESBL										9	7	9
E. faecalis	Aminopenicillins I+R	13	5	4	5	6	3	2	5	5	1	5	9
	HL Aminoglycosides R	50	40	28	35	31	37	37	46	37	33	39	35
	Glycopeptides R	3	<1	<1	<1	<1	<1	<1	<1	<1	1	<1	<1
E. faecium	Aminopenicillins I+R	100	56	47	69	82	69	78	79	82	98	98	90
	HL Aminoglycosides R	100	67	41	63	62	59	59	65	60	66	61	55
	Glycopeptides R	<1	22	6	3	6	3	2	6	12	2	0	7
K. pneumoniae	Aminoglycosides R					38	33	38	51	49	43	45	51
	Fluoroquinolones R					18	23	34	44	48	43	43	45
	3. gen Cef R					46	34	40	54	56	50	44	50
	ESBL										51	52	50
	Carbapenems I+R										<1	<1	1
P. aeruginosa	Piperacillin R					25	38	30	34	23			
	Piperacllin/Tazobactam R									16	23	18	23
	Ceftazidime R					6	11	14	13	12	17	14	20
	Carbapenems R					24	25	26	30	26	30	21	25
	Aminoglycosides R					35	47	40	39	26	34	26	24
	Fluoroquinolones R					34	35	30	33	27	34	24	23
A. baumanii	Carbapenems R												91

Tablica-*Table 3.*
Prikaz invazivnih, gram-pozitivnih izolata u 2013.g. prema demografskim podacima pacijenata
Selected details on invasive gram-positive isolates from the reporting period 2013

	S.pneumoniae		***S.aureus***		***Enterococcus* spp.**	
	n=119		**n=533**		**n=250**	
	% tot	**% PNPS**	**% tot**	**% MRSA**	**% tot**	**% VRE**
UZORAK ***SAMPLE***						
Krv / Blood	86	24	99	24	100	2
Likvor / CSF	13	40	<1	0	0	0
SPOL ***GENDER***						
M	47	29	63	25	62	3
Ž / F	50	24	34	24	35	3
Nepoznato / Unknown	3	0	4	0	3	0
DOB ***AGE***						
0-4	29	37	3	6	7	0
5-19	6	29	2	33	<1	0
20-64	31	14	36	21	34	7
>65	34	25	57	27	56	0
Nepoznato / Unknown	0	0	0	0	2	0
ODJEL ***DEPARTMENT***						
Intenzivna / ICU	5	45	10	43	9	0
Interna / Medical	13	21	28	44	64	4
Kirurgija / Surgery	0	0	5	52	15	0
Nepoznato / Unknown	80	0	57	8	12	0

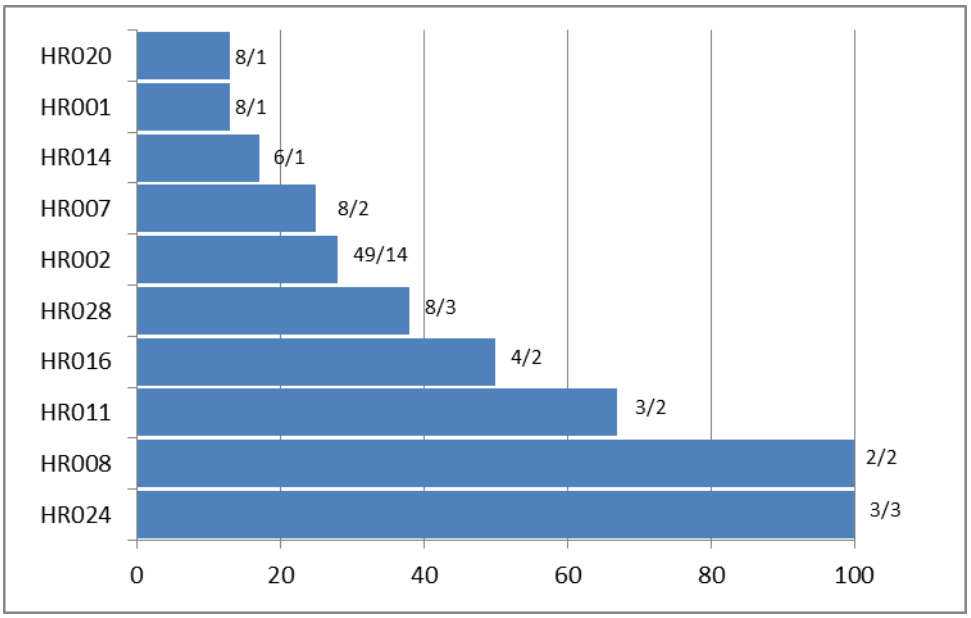
PNSP=Penicillin Non-Susceptible *S. Pneumoniae* MRSA=Methicillin Resistant *S.aureus* VRE=Vancomycin Resistant Enterococcus

Tablica-*Table 4.*
Prikaz invazivnih, gram-negativnih izolata u 2013.g. prema demografskim podacima pacijenata
Selected details on invasive, gram-negative isolates from the reporting period 2013

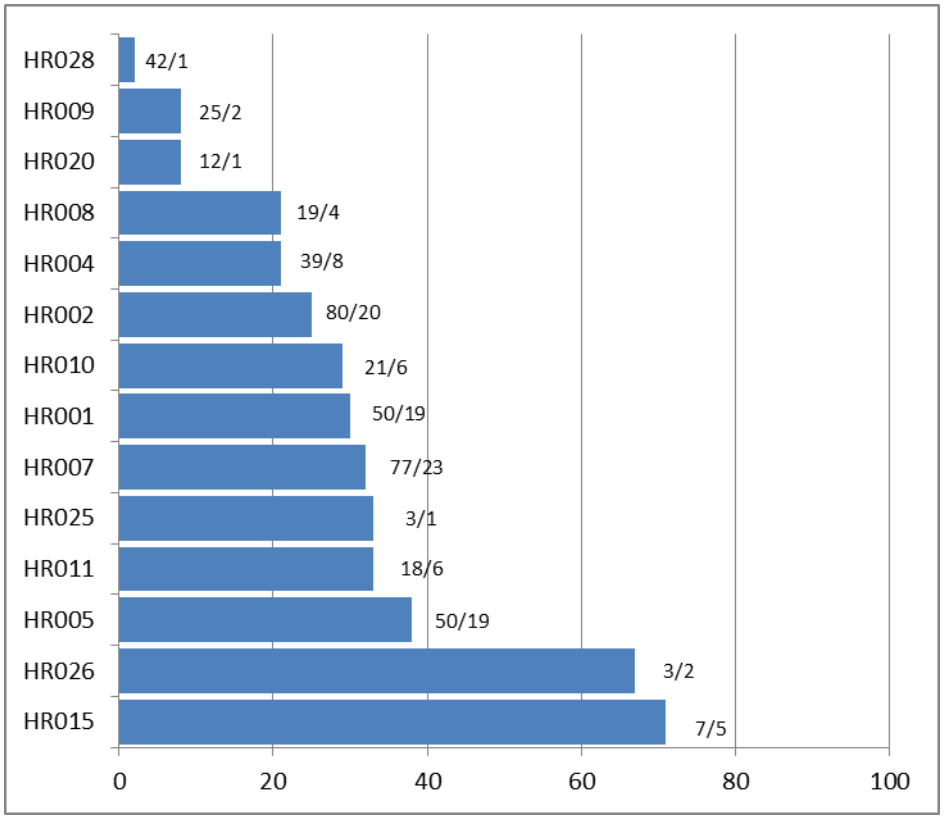
	E.coli			***Acinetobacter* spp.**		***K.pneumoniae***		***P.aeuroginosa***	
	n=1066			**n=114**		**n=396**		**n=256**	
	% tot	**% FREC**	**% CREC**	**% tot**	**% CRA**	**% tot**	**% CRKP**	**% tot**	**% CRPA**
UZORAK ***SAMPLE***									
Krv / Blood	99	21	7	96	87	100	50	98	24
Likvor / CSF	<1	0	0	4	75	0	0	2	80
SPOL ***GENDER***									
M	40	31	9	61	90	55	52	57	29
Ž / F	57	17	5	35	85	41	46	38	20
Nepoznato / Unknown	3	31	15	4	60	4	53	5	27
DOB ***AGE***									
0-4	3	5	3	6	0	11	69	4	30
5-19	1	0	0	1	100	<1	100	5	38
20-64	33	20	7	37	90	38	51	34	34
>65	62	21	7	56	95	50	44	57	19
Nepoznato / Unknown	1	0	0	0	0	0	0	0	0
ODJEL ***DEPARTMENT***									
Intenzivna / ICU	4	26	13	38	100	14	64	15	28
Interna / Medical	80	20	7	36	76	64	42	52	25
Kirurgija / Surgery	8	30	13	14	100	13	64	16	14
Nepoznato / Unknown	8	11	0	12	71	9	0	17	0

FREC=Fluoroquinolone Resistant *E.coli* CREC=3rd gen. Cepfalosporine Resistant *E.coli* CRKP=3rd gen. Cepfalosporine Resistant *K. pneumoniae*
CRPA=Carbapenem Resistant *P. aeruginosa* CRA=Carbapenem Resistant *Acinetobacter* spp.

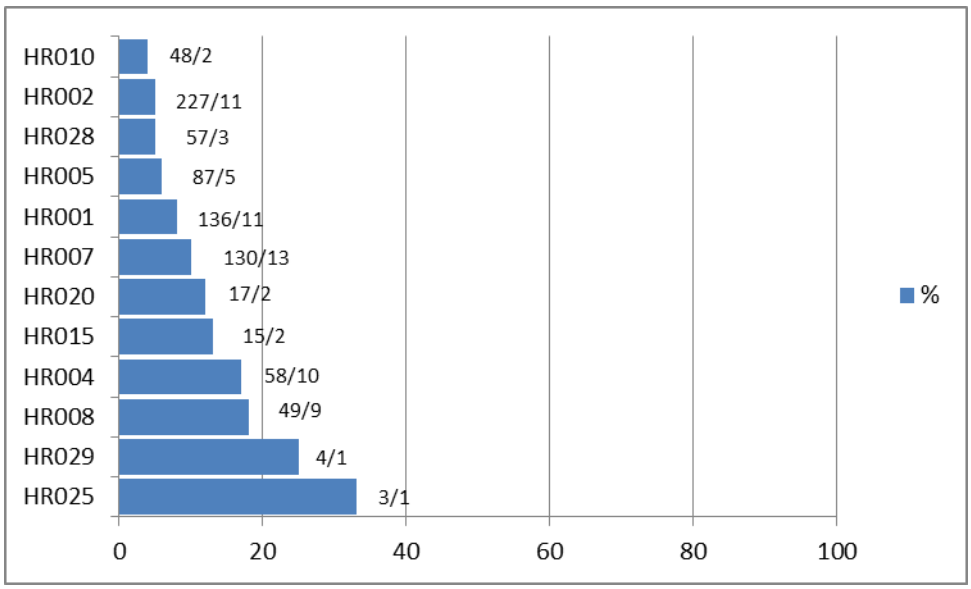
Slika-*Figure 1.*
Udio (%) izolata *S. pneumoniae* smanjene osjetljivosti na penicilin (PNSP) po laboratorijima
Proportion (%) of penicillin non-susceptible S. pneumoniae (PNSP) by laboratory



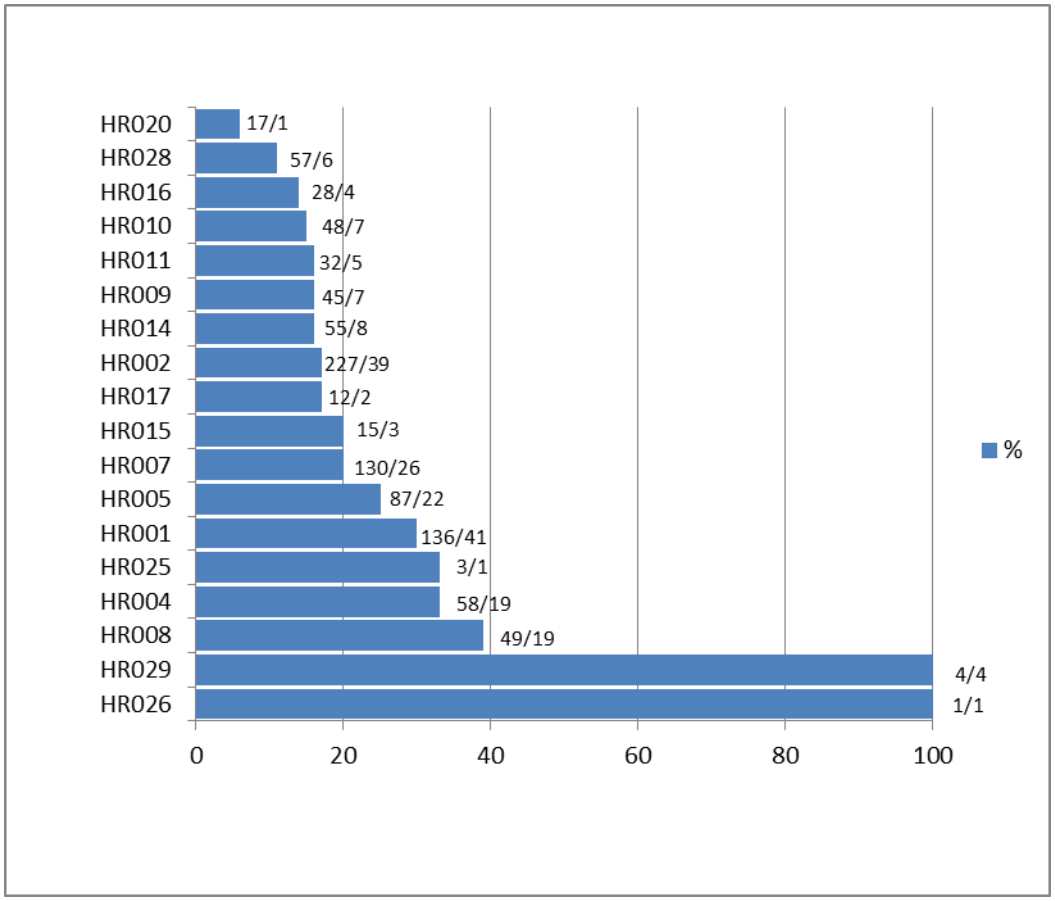
Slika-*Figure 2.*
Udio (%) MRSA izolata po laboratorijima
Proportion (%) of MRSA isolates by laboratory



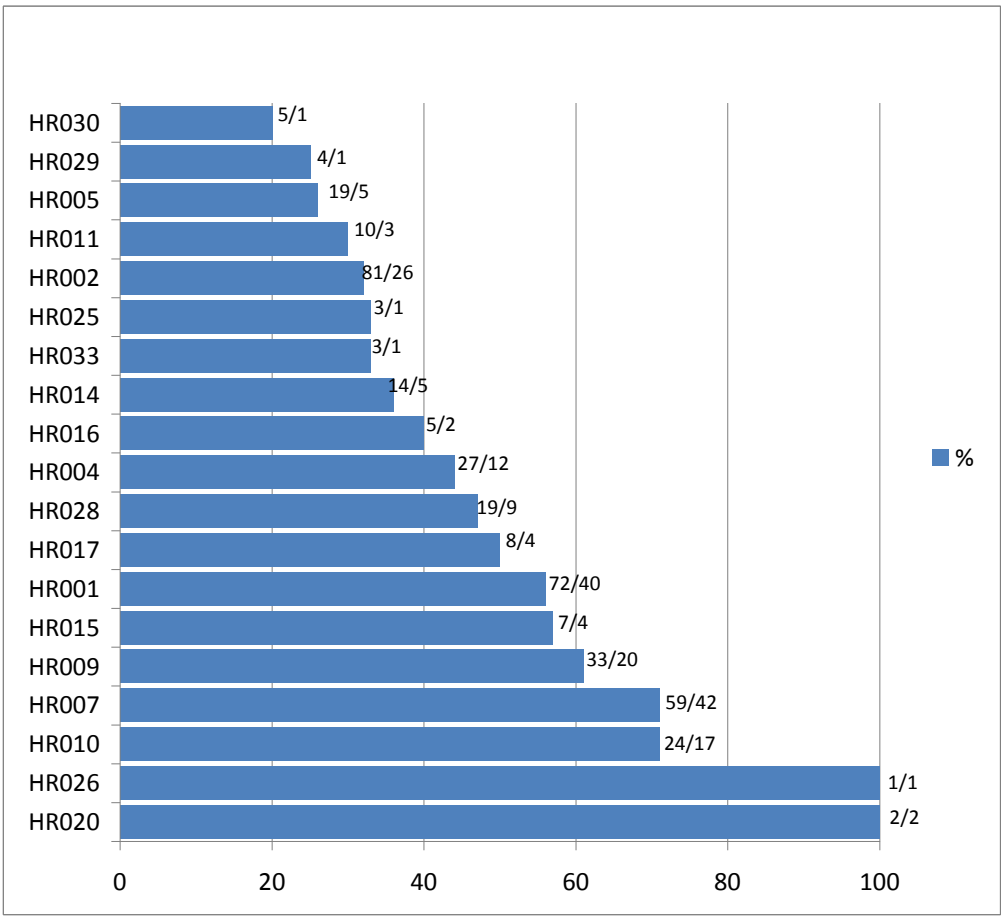
Slika-*Figure 3.*
Udio (%) ceftazidim rezistentnih izolata *E. coli* (CREC) po laboratorijima
Proportion (%) of ceftazidime resistant E. coli isolates (CREC) by laboratory



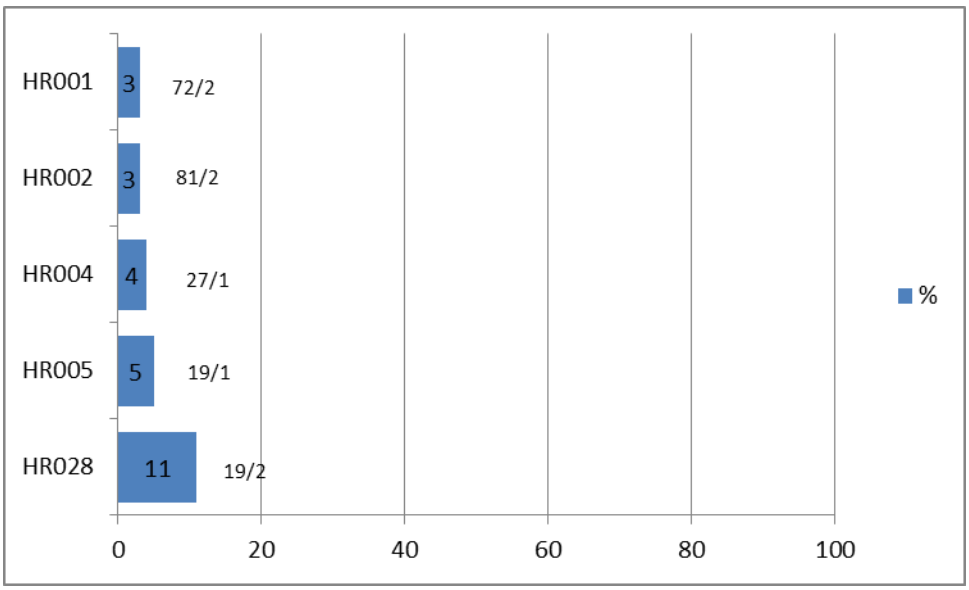
Slika-*Figure 4.*
Udio (%) fluorokinolon rezistentnih izolata *E. coli* (FREC) po laboratorijima
Proportion (%) of fluoroquinolone resistant E.coli isolates (FREC) by laboratory



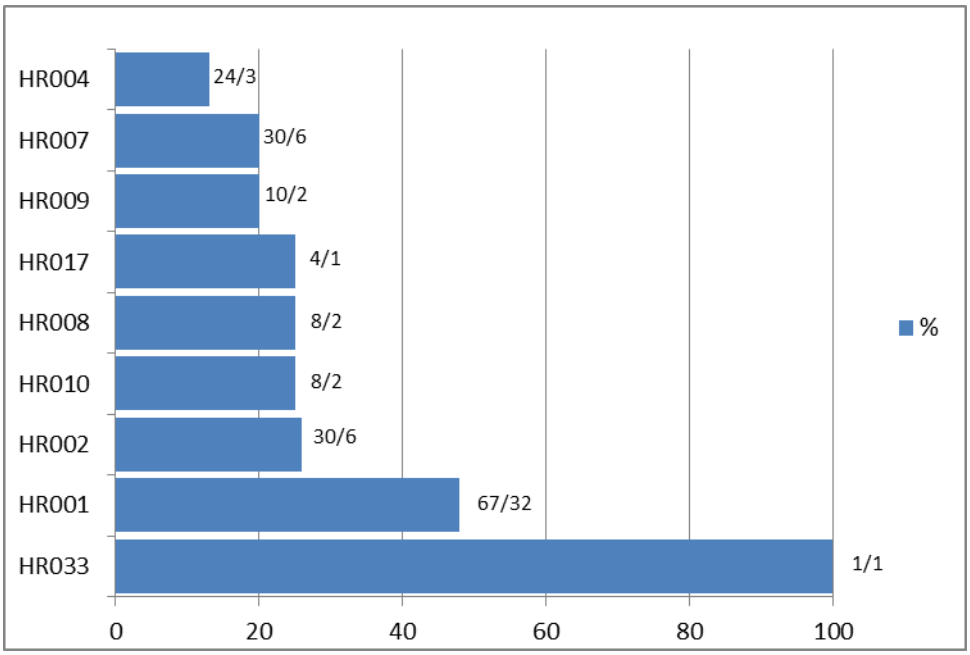
Slika-*Figure 5.*
Udio (%) ceftazidim rezistentnih izolata *K. pneumoniae* (CRKP) po laboratorijima
Proportion (%) of ceftazidime resistant K. pneumoniae (*CRKP) by laboratory*



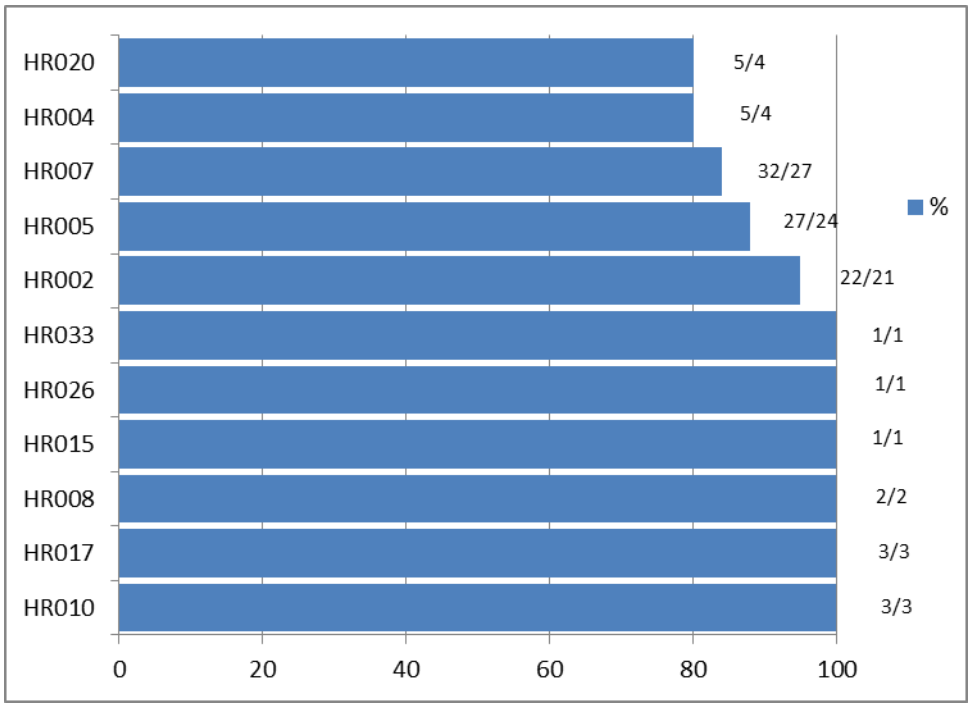
Slika-*Figure 6.*
Udio (%) karbapenem rezistentnih izolata *K. pneumoniae* (CRKP) po laboratorijima
Proportion (%) of carbapenem non-susceptible K. pneumoniae (*CRKP) by laboratory*



Slika-*Figure 7.*
Udio (%) karbapenem rezistentnih izolata *P. aeruginosa* (CRPA) po laboratorijima
Proportion (%) of carbapenem resistant P. aeruginosa (CRPA) by laboratory



Slika-*Figure 8.*
Udio (%) karbapenem rezistentnih izolata *Acinetobacter* spp. po laboratorijima
Proportion (%) of carbapenem resistant Acinetobacter spp. *by laboratory*



POGLAVLJE/CHAPTER 4.

POTROŠNJA ANTIBIOTIKA U HRVATSKOJ
ANTIBIOTIC CONSUMPTION IN CROATIA

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Potrošnja antibiotika u Hrvatskoj
Antibiotic consumption in Croatia

Izvanbolnička potrošnja antibiotika

Praćenje potrošnje antibiotika u Hrvatskoj započelo je 2001. godine u okviru European Surveillance of Antibiotic Consumption (ESAC). Sve zemlje uključene u ESAC, pa tako i Hrvatska, koristile su istu metodologiju u prikupljanju podataka.

Podaci o potrošnji antibiotika (J01) se prikupljaju u skladu s anatomsko-terapijsko-kemijskom klasifikacijom (ATK) na petoj razini, a objavljuju na četvrtoj i trećoj razini prema klasifikaciji, odvojeno za bolničku i izvanbolničku potrošnju.

Prikupljeni podaci se unose u ABC kalkulator, koji je ažuriran i usklađen s hrvatskim tržištem. Potrošnja antibiotika se izražava u definiranim dnevnim dozama na 1000 stanovnika po danu (DDD/TID).

Do 2012. godine obrađeni su podaci o ambulantnoj potrošnji antibiotika dobiveni samo od veledrogerija. U 2012. godini, po prvi puta, su prikupljeni i obrađeni podaci o ambulantnoj potrošnji antibiotika i od Hrvatskog zavoda za zdravstveno osiguranje (HZZO), odnosno antibiotici izdani na propisani („crveni“) recept. S obzirom da je HZZO jedina velika osiguravajuća tvrtka u Hrvatskoj i da se svi antibiotici izdaju na recept, HZZO podaci se mogu smatrati vjerodostojnim podacima potrošnje antibiotika u Hrvatskoj. U zadnje dvije godine praćenja ambulantne potrošnje antibiotika raspolažemo s podacima dobivenim iz dva izvora (veledrogerija, HZZO). Podaci dobiveni od HZZO-a se smatraju službenim podacima za Hrvatsku i od 2012.g. ti se podaci koriste u praćenju izvanbolničke potrošnje antibiotika (tablica 1, slika 1).

Prilikom izračunavanja potrošnje antibiotika za 2012. godinu, također po prvi puta, kao denominator je upotrebljen broj stanovnika prema popisu stanovništva iz 2011. godine (4 284 889), što je manji broj u odnosu na prethodni popis stanovništva iz 2001. godine (4 555 219). Od tada se kao denominator koristi taj broj stanovnika što je moglo djelomično utjecati na podatke izražene u DDD/TID.

U 2013. godini ambulantna potrošnja iznosi 21,1 DDD/TID, što ukazuje na smanjenje potrošnje u komparaciji s prethodnom godinom (21,72 DDD/TID), kada su korišteni isti denominator i isti izvor podatka (HZZO). Uspoređujući potrošnju antibiotika ovisno o izvoru podataka (veledrogerije i HZZO) u 2013. godini, isto kao i u prethodnoj godini zabilježena je manja potrošnja prema podacima dobivenim od HZZO-a (20,10 DDD/TID) u odnosu na veledrogerije (22,08 DDD/TID) kako broja potrošenih DDD-a tako i ukupna potrošnja izražena u DDD/TID (tablica 3, slika 2).

Na tablici 4 uočava se razlika u potrošnji pojedinih klasa antibiotika ovisno o izvoru. Jedan od razloga uočene razlike u potrošnji antibiotika je kupovina antibiotika na privatni recept te direktno naručivanje antibiotika putem veledrogerija od strane liječnika primarne zdravstvene zaštite za potrebe liječenja svojih bolesnika. Najveća razlika se bilježi u klasi penicilna J01C (0,65 DDD/TID), dok je u drugim klasama ta razlika vrlo mala i kreće se od 0,01 do 0,19 DDD/TID (tablica 4; slika 3) .

Trend pada izvanbolničke potrošnje antibiotika uočava se kod oba izvora podataka (HZZO, veledrogerije). U 2013. godini bilježi se pad potrošnje kod svih klasa antibiotika, osim klase J01CA (penicilini širokog spektra), kod koje je uočljiv porast sa 2,96 na 3,0 DDD/TID. Posebno je povoljan podatak da je smanjena potrošnja širokospektralnih antibiotika, cefalosporina i kombinacija penicilina s inhibitorima beta-laktamaza. Potrošnja fluorokinolona je također, u laganom padu, dok je potrošnja nitrofurantoina identična u 2012. i 2013. godini (tablica 1).

Ohrabruje uočen trend pada potrošnje antibiotika u 2013. godini, te se nadamo njegovom nastavku i u sljedećoj godini. Uočenu razliku u potrošnji antibiotika ovisno o izvoru nastojat ćemo rasvjetliti na način da se istraži udio „privatnih“ recepata koji se pojavljuju u ljekarnama u odnosu na one koji prolaze preko evidencije HZZO-a.
Izvanbolnička potrošnja antibiotika u 2013. godini čini 92% potrošnje, potpuno identično kao i prošle godine. Nadamo se da je niža izvanbolnička potrošnja antibiotika početak željenog trenda smanjene potrošnje antibiotika.

Outpatient antibiotic consumption

Monitoring of antibiotic consumption in Croatia started in 2001 within the European Surveillance of Antibiotic Consumption (EASC) project. Each ESAC member country, including Croatia, used the same methodology for collecting the data.

The data on antibiotic consumption (J01) is being collected at the fifth level in accordance with the Anatomical Therapeutic Chemical classification (ATC), and the results are being published on the fourth and the third level, separately for hospital and outpatient consumption.

Collected data is entered in the ABC calculator, which is regularly updated and adjusted to Croatian market. The antibiotic consumption is expressed in defined daily doses per 1000 inhabitants per day (DDD/TID).

Until 2012 wholesales data were used for ambulatory care consumption. In 2012, for the first time, outpatient data provided by the Croatian Health Insurance Fund (CHIF) were used. As CHIF is the single major health insurance company in Croatia and all antibiotics are reimbursed, CHIF data are considered to be reliable data for outpatient consumption in Croatia. In the past two years of analysing the outpatient antibiotic consumption we had two data sources on disposal (wholesales, CHIF). CHIF data are considered official consumption data for Croatia and are used in surveillance of outpatient antibiotic consumption since 2012 (table 1, figure 1).

Also, in 2012 for the first time we used the census of 2011 as a denominator (4 284 889), which is a smaller number compared to the previous census in 2001 (4 555 219). From year 2012 on we use the new denominator which might slightly influence DDD/TID data.

In 2013 the ambulatory antibiotic consumption was 21,1 DDD/TID, which indicates a decrease in consumption compared to the previous year (21,72 DDD/TID), when the same denominator and the same data source were used.

Comparing the antibiotic consumption depending on the data source (wholesalers and CHIF) in 2013, just like in the previous year, a lower consumption was recorded by the CHIF data (20,10 DDD/TID), in comparison to the wholesalers (22,08 DDD/TID), both for the DDD consumption and the total consumption expressed in DDD/TID (table 3, figure 2).

A difference in consumption of antibiotic classes was noticed depending on the source of data (table 4). Reasons for the observed difference in antibiotic consumption may be purchasing antibiotics on a private prescription or direct ordering of antibiotics from wholesalers by primary care physicians. The greatest difference is recorded in the penicillins class J01C (0,65 DDD/TID), while in other classes, the difference is very small, ranging from 0, 01 to 0, 19 DDD/TID (table 4, figure 3).

The declining trend of outpatient antibiotic consumption is recorded regardless of the data source (CHIF, wholesalers). In 2013 there was a decrease in the consumption of all classes of antibiotics, except for the class J01C (penicillins of a broad spectrum), in which we can notice the increase from 2.96 to 3, 0 DDD/TID. It is especially positive that the use of broad spectrum antibiotics like cephalosporins and penicillins with beta-lactamase inhibitors is decreasing. Consumption of the fluoroquinolones is also slightly decreasing while the nitrofurantoin consumption was identical in 2012 and 2013 (table 1).

Observed decrease in antibiotic consumption in 2013 is encouraging and we hope that this trend is going to continue in the following year. We will try to clarify the observed differences in the antibiotic consumption depending on the data source by examining the proportion of ‘private’ recipes.

The outpatient antibiotic consumption in 2013 makes 92% of the total consumption, exactly the same as the last year. We hope that the lower outpatient antibiotic consumption is a beginning of a desired decreasing trend of antibiotic consumption.

Tablica-*Table 1.*
Izvanbolnička potrošnja antibiotika (DDD/TID)
Ambulatory antibiotic consumption (DDD/TID)

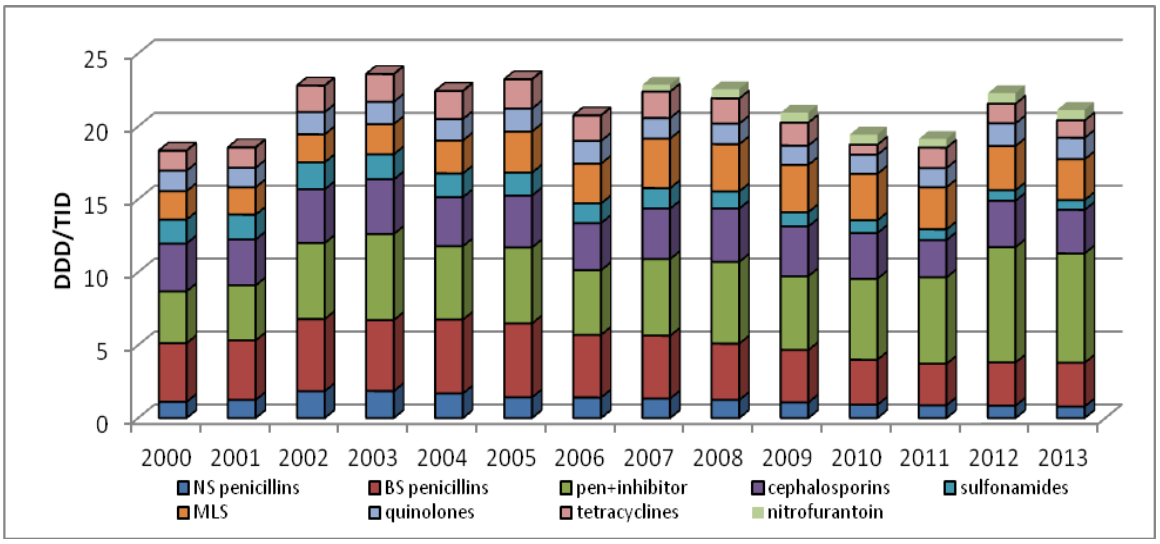
ATC šifra ATC code	ANTIBIOTIK ANTIBIOTIC	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012*	2013*
JO1AA	Tetraciklini Tetracylines	1.90	1.91	2.01	1.74	1,81	1,73	1,57	1,46	1,39	1,35	1,19
JO1CA	Penicilini širokog spektra Broad spectrum penicillins	4.82	5.10	5.07	4.30	4,31	3,86	3,60	3,09	2,84	2,96	3,00
JO1CE	Penicilini uskog spektra Narrow spectrum penicillins	1.85	1.71	1.42	1.41	1,34	1,24	1,07	0,91	0,88	0,85	0,79
JO1CF	Beta-laktamaza rezistentni penicilini Beta-lactamase resistant penicillins	0.06	0.06	0.05	0.05	0,05	0,04	0,00	0,00	0,00	0,00	0,00
JO1CR	Kombinacije s beta-laktamaza inhibitorima	5.9	5.04	5.21	4.43	5,26	5,61	5,06	5,55	5,93	7,91	7,50
JO1DA	Cefalosporini I gen. I gen. cephalosporins	1.94	1.87	1.85	1.66	1,88	1,56	1,21	1,05	0,84	0,82	0,77
	Cefalosporini II gen. II gen. cephalosporins	1.37	1.19	1.29	1.15	1,02	1,55	1,59	1,50	1,19	1,80	1,77
	Cefalosporini III gen. III gen. cephalosporins	0.44	0.39	0.42	0.42	0,56	0,55	0,61	0,59	0,53	0,57	0,45
JO1EE	Sulfonamides + trimethoprim	1.72	1.64	1.57	1.35	1,4	1,17	0,98	0,87	0,73	0,72	0,67
JO1F	Macrolides, lincosamides	2.07	2.27	2.82	2.73	3,40	3,24	3,24	3,19	2,89	3,03	2,80
JO1G	Aminoglycosides	0.01	0.01	0.01	0.01	0,01	0,01	0,01	0,01	0,01	0,01	0,00
JO1MA	Fluoroquinolones	1.53	1.47	1.57	1.56	1,41	1,41	1,33	1,31	1,32	1,55	1,47
JO1XE	Nitrofurantoin					0,47	0,63	0,68	0,69	0,60	0,72	0,72
UKUPNO / TOTAL		**23.61**	**22.66**	**23.29**	**20,81**	**22,92**	**22,60**	**20,95**	**20,22**	**19,16**	**21,72**	**21,10**

***** Izvor podataka Hrvatski zavod za zdravstveno osiguranje / origin of data Croatian Health Insurance Fund
Popis stanovništva 2011/ The Croatian Bureau of Statistics, Census 2011

Tablica-*Table 2.*
Bolnička potrošnja antibiotika (DDD/TID)
Hospital antibiotic consumption (DDD/TID)

ATC šifra ATC code	ANTIBIOTIK ANTIBIOTIC	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
JO1AA	Tetracylines	0.15	0.08	0.09	0.07	0,06	0,06	0,06	0,05	0,07	0,06	0,05
JO1CA	Penicilini širokog spektra Broad spectrum penicillins	0.33	0.15	0.15	0.12	0,09	0,08	0,05	0,04	0,06	0,06	0,09
JO1CE	Penicilini uskog spektra Narrow spectrum penicillins	0.35	0.20	0.14	0.12	0,10	0,06	0,01	0,01	0,04	0,03	0,03
JO1CF	Beta-laktamaza rezistentni penicilini Beta-lactamase resistant penicillins	0.04	0.03	0.03	0.03	0,04	0,02	0,00	0,00	0,03	0,04	0,03
JO1CR	Kombinacije s beta-laktamaza inhibitorima	0.79	0.40	0.36	0.27	0,22	0,25	0,23	0,22	0,51	0,52	0,45
JO1DA	Cefalosporini I gen. cephalosporins	0.17	0.09	0.11	0.10	0,11	0,09	0,10	0,09	0,11	0,10	0,08
	Cefalosporini II gen. cephalosporins	0.19	0.27	0.25	0.22	0,22	0,19	0,15	0,21	0,23	0,23	0,21
	Cefalosporini III + IV gen. cephalosporins	0.12	0.09	0.12	0.11	0,13	0,14	0,16	0,16	0,16	0,15	0,16
JO1DH	Carbapenems	0.02	0.02	0.02	0.02	0,04	0,04	0,04	0,04	0,07	0,07	0,06
JO1EE	Sulfonamides + trimethoprim	0.20	0.09	0.08	0.07	0,07	0,06	0,06	0,05	0,05	0,06	0,04
JO1F	Macrolides, lincosamides	0.16	0.10	0.12	0.10	0,11	0,11	0,12	0,11	0,15	0,16	0,15
JO1G	Aminoglycosides	0.12	0.10	0.11	0.10	0,09	0,10	0,10	0,09	0,12	0,11	0,10
JO1MA	Fluoroquinolones	0.22	0.15	0.18	0.17	0,19	0,19	0,21	0,21	0,23	0,22	0,22
JO1XA	Glycopeptides	0.02	0.02	0.02	0.02	0,03	0,03	0,03	0,03	0,04	0,03	0,03
JO1XD	Metronidazole	0.06	0.01	0.06	0.05	0,06	0,06	0,07	0,07	0,07	0,07	0,08
JO1XE	Nitrofurantoin					0,01	0,01	0,01	0,01	0,01	0,02	0,01
UKUPNO / TOTAL		**2.94**	**1.80**	**1.84**	**1.57**	**1,57**	**1,49**	**1,40**	**1,39**	**1,96**	**1,98**	**1,80**

Slika-*Figure 1*.
Izvanbolnička potrošnja antibiotika u Hrvatskoj, 2000. – 2013.
Ambulatory antibiotic consumption in Croatia, 2000 – 2013

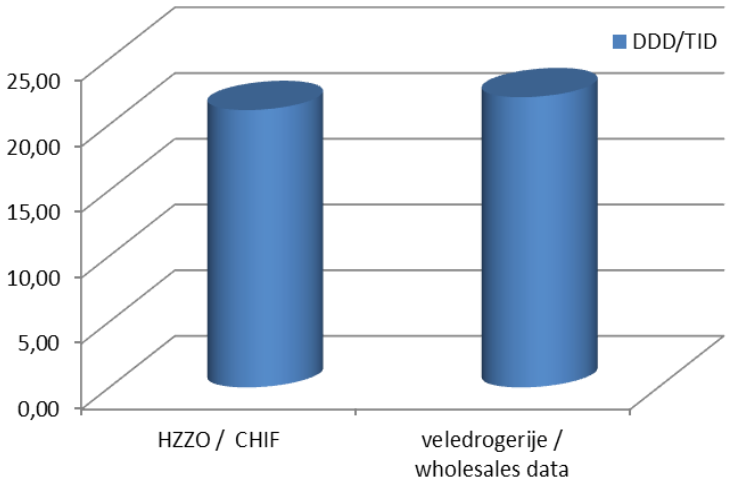


NS penicillins = penicilini uskog spektra; BS penicillins = penicilini širokog spektra; Pen+inhibitor = penicilini s inhibitorima; Cephalosporins = cefalosporini; Sulfonamides = sulfonamidi; MLS = makrolidi, linkozamidi, streptogramini; Quinolones = kinoloni; Tetracyclines = tetraciklini

Tablica-*Table 3.*
Ambulantna potrošnja antibiotika (DDD/TID) usporedba podataka HZZO i veledrogerija
Ambulant antibiotic consumption (DDD/TID) comparison between CHIF data and wholesales data

	HZZO CHIF	**veledrogerije wholesales data**
DDD	32775118,1	34531182,4
TID	21,10	22,08

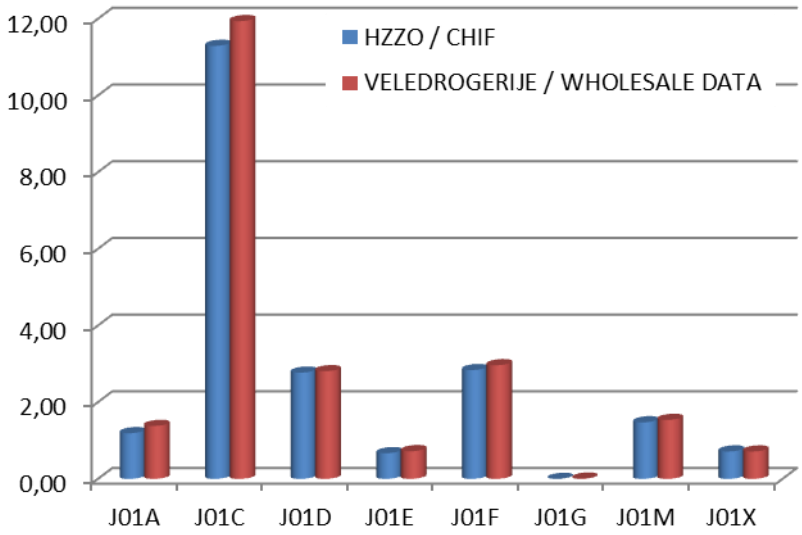
Slika-*Figure 2.*
Ambulantna potrošnja antibiotika (DDD/TID) usporedba podataka HZZO i veledrogerija
Ambulant antibiotic consumption (DDD/TID) comparison between CHIF data and wholesales data



Tablica-*Table 4.*
Ambulantna potrošnja antibiotika (DDD/TID) po klasama, usporedba podataka HZZO i veledrogerija
Ambulant antibiotic consumption (DDD/TID) *by class, comparison between CHIF data and wholesales data*

DDD/TID	HZZO CHIF	veledrogerije wholesales data
J01A	1,19	1,38
J01C	11,29	11,94
J01D	2,77	2,81
J01E	0,67	0,72
J01F	2,84	2,96
J01G	0,00	0,01
J01M	1,47	1,54
J01X	0,72	0,71

Slika-*Figure 3.*
Ambulantna potrošnja antibiotika (DDD/TID) po klasama, usporedba podataka HZZO i veledrogerija
Ambulant antibiotic consumption (DDD/TID) *by class, comparison between CHIF data and wholesales data*



Potrošnja antibiotika u hrvatskim bolnicama

Kao i prethodnih godina, u 2013. godini su prikupljeni podaci o bolničkoj potrošnji antibiotika - skupina J01A, u skladu s ATK klasifikacijom. Podaci su prikupljeni u paketićima / ampulama i uneseni u ABC kalkulator, koji je usklađen s hrvatskim tržištem.

Za iskazivanje potrošnje antibiotika neophodni su i administrativni podaci, koji se prikupljaju od svake bolnice na zasebnom formularu (broj bolničkoopskrbnihih dana, broj primitaka, broj kreveta, broj dječjih kreveta, broj JIL-ova).

Zadnje tri godine prikupljaju se podaci i za dnevnu bolnicu (broj terapijskih dana), što omogućuje objektivniji prikaz potrošnje antibiotika u odnosu na aktivnosti bolnice.

Do 2006. godine obrađivani su samo podaci o bolničkoj potrošnji antibiotika dobiveni putem veledrogerija. Osnutkom Interdisciplinarne sekcije za kontrolu rezistencije bakterija na antibiotike (ISKRA) započelo se s praćenjem bolničke potrošnje i iz drugog izvora, tj. prikupljanje podataka iz bolničkih ljekarni.

Prikupljanjem podataka o bolničkoj potrošnji antibiotika od bolničkih ljekarni omogućeno je izračunavanje potrošnje antibiotika te izražavanje potrošnje u definiranim dnevnim dozama (DDD) na 100 bolničkih dana (BOD), što je mnogo precizniji pokazatelj potrošnje u odnosu na prikazivanje potrošnje izražene na 1000 stanovnika po danu (TID), kako je bila praksa do 2006. godine.

Kroz sve godine praćenja potrošnje antibiotika iz dva izvora (bolničke ljekarne, veledrogerije) uočava se razlika u ukupnoj potrošnji i po klasama antibiotika (tablica 5, slika 4) pa je tako i u 2013. uočena ista pojava kako u ukupnoj potrošnji, tako i u potrošnji različitih klasa antibiotika. Najveća razlika je uočena kod penicilina (J01C), zatim aminoglikozida (J01G); skupine ostalih antibiotika (J01X) te makrolida (J01F) u korist veće potrošnje prema podacima dobivenim iz bolničkih ljekarni. Prema podacima dobivenim od veledrogerija veća potrošnja se bilježi kod klase J01D te makrolid-linkozamid-streptogramin (J01F). Razlozi tih razlika nisu u potpunosti jasni.

U 2014. godini podatke o bolničkoj potrošnji bilo je moguće poslati i elektronskim putem na adresu iskra.antibiotici@gmail.com uz dosadašnji način slanja na CD-u poštom na adresu Ministarstva zdravlja. Razlog je jednostavnost i vremensko skraćivanje procesa prikupljanja podataka, a time i obrade te provjere rezultata. Kao redovita praksa uhodano je slanje obrađenih podataka na provjeru u svaku ustanovu uz mogućnost komparacije potrošnje u svim prethodnim godinama praćenja za dotičnu bolničku ustanovu.

Usporednim praćenjem potrošnje antibiotika iz dva izvora (veledrogerije i bolničke ljekarne) prati se razlika u potrošnji, koja u 2013. godini iznosi 0,09 TID, što je najmanja zabilježena razlika do sada (tablica 5, slika 4).

U odnosu na prethodnu godinu bolnička potrošnja bilježi pad (1,8 DDD/TID) prema podacima dobivenim iz bolničkih ljekarni (tablica 5, slika 4), odnosno 40,10 DDD/100 BOD ako se kao denominator koriste bolničko opskrbni dani (tablica 6, slika 5). Pad potrošnje se uočava u svim klasama, osim kinolona (J01M), koji bilježe porast (s 4,66 na 5,00 DDD/ 100 BOD) i klase J01X (ostali antibiotici) s 2,82 na 3,05 DDD/100 BOD (tablica 7, slika 6).

Klasa J01A (tetraciklini) zadnje tri godine bilježi pad potrošnje. Uz nju i klasa J01E (sulfonamidi) kao najmanje zastupljena klasa u potrošnji antibiotika, pokazuje silazni trend.

Klasa J01C (penicilini) i J01D (cefalosporini) pokazuju silazni trend u potrošnji zadnje tri godine. U 2013. godini uočava se najmanja razlika u potrošnji između te dvije najzastupljenije klase (0,73 / 100 BOD), čija potrošnja zajedno čini preko 60% ukupne potrošnje antibiotika (tablica 7, slika 6).

Kod klase J01F (makrolid, linkozamid, streptogramin) i klase J01G (aminoglikozidi) uočava se trend pada potrošnje od 2011. godine.

U 2013. godini porast potrošnje uočljiv je kod kinolona (J01M) te kod klase J01X, koja je, po prvi puta od početka praćenja potrošnje antibiotika, premašila vrijednost od 3 DDD/100BOD (tablica 7, slika 6).

U 2013. godini pratila se potrošnja antibiotika u 13 **kliničkih ustanova** (tablica 8).
Iako je reorganizacijom bolničkog sustava došlo do spajanja više različitih ustanova u jednu, kod nekih se nastavilo sa praćenjem kao zasebne ustrojstvene jedinice zbog profila same ustanove i mogućnosti kvalitetnije analize potrošnje antibiotika. Kliničke ustanove K10 i K12 su pripojene većim kliničkim centrima. Obradom podataka o potrošnji antibiotika u 13 kliničkih ustanova, uočava se veliki raspon u potrošnji (od 18,4 do 140,5 DDD/100 BOD). Tako velike razlike proizlaze iz profila kliničkih ustanova i nisu sve međusobno usporedive. Svaka bolnica za sebe može pratiti trendove u ukupnoj potrošnji, kao i strukturu potrošnje, što je koristan indikator kvalitete propisivanja antibiotika. Osam klinika (K01; K04; K05; K06; K08; K11; K13; K14) bilježi pad u potrošnji antibiotika u odnosu na godinu prije. Osobito je dojmljiv pad u potrošnji antibiotika klinike 6 (K06), koji je zbog velike vrijednosti dodatno provjeren i potvrđen kao ispravan podatak. Kao razlog je navedeno uvođenje posebnih mjera i strogo kontolirani režim propisivanja antibiotika, osobito određenih klasa što je rezultiralo značajnim padom potrošnje (slika 7). U pet klinika (K02; K03; K07; K09; K15) uočava se porast potrošnje. Najveći skok u porastu potrošnje bilježi se za kliniku K09 (za 9,6 DDD/BOD), dok je u drugim klinikama porast diskretan. Na slici 7 se dobro uočavaju trendovi u potrošnji antibiotika za svaku kliničku ustanovu.

Potrošnja antibiotika u skupini **općih bolnica** se kreće od 24,1 do 72,4 DDD/100 BOD, što ukazuje na velike razlike u propisivanju antibiotika u ovoj najhomogenijoj skupini bolnica (tablica 9). Trećina općih bolnica (7) troši u rasponu od 41 – 50 DDD/100 BOD. Podjednaki broj bolnica (6) troši u rasponu od 51-60 DDD/100 BOD i od 61-70 DDD/BOD. Dvije opće bolnice (07; 020) najveći su potrošači među općim bolnicama s potrošnjom iznad 70 DDD/100 BOD (tablica 9). Od 22 opće bolnice u 13 se uočava pad potrošnje antibiotika (01; 03; 04; 07; 08; 012: 015; 017; 018; 019; 022; 023; 024). Osobito veliki pad potrošnje bilježe dvije bolnice 01 (za 16,7 DDD/100 BOD) i 04 (za 14,4 DDD/100 BOD). Kod ostalih bolnica pad u potrošnji je mnogo diskretniji. Bolnica 02 u skupini je s bolnicama koje troše od 41 do 50 DDD/100 BOD i svih sedam godina po potrošnji se kreće unutar tih raspona, a zadnje dvije godine pokazuje gotovo identičnu potrošnju. Bolnica 021 i dalje pokazuje kontinuirani trend porasta potrošnje, kao i prethodnih godina (slika 8).

Psihijatrijske bolnice kao ustanove s najnižom potrošnjom antibiotika kreću se po potrošnji u rasponu od 4,9 do 14,2 DDD/100BOD (tablica 10). U 2013. godini ističe se psihijatrijska bolnica 09 po osobitom skoku u potrošnji antibiotika. Radi se o bolnici najmlađoj po osnutku i uključenosti u praćenje potrošnje antibiotika. Potrošnja antibiotika kod ostalih psihijatrijskih

bolnica je uglavnom slična, osim 02 kod koje je uočen značajan pad u potrošnji. Psihijatrijska bolnica 05 zaustavila je trend porasta potrošnje antibiotika u 2013. godini (slika 9).

Specijalne bolnice su podijeljene u dvije velike grupe s obzirom na njihov profil rada i kao takve bilježe veliki raspon u potrošnji antibiotika. U prvoj skupini nalazi se 11 bolnica, koje su namijenjene liječenju (akutnom/kroničnom), dok je u drugoj skupini 12 ustanova namijenjeno rehabilitaciji. U prvoj skupini ustanova raspon potrošnje se kreće od 13,1 do 61,5 DDD/100 BOD. U drugoj skupini kretanje potrošnje antibiotika je od 0,7 do 14,2 DDD/100 BOD (tablica 11, slika 10).

Antibiotici su skupina lijekova od iznimno velikog značaja za suvremenu medicinu, stoga je praćenje potrošnje antibiotika od osobite važnosti. Loše odabrana i krivo primijenjena antibiotska terapija potiče nastanak otpornosti kod bakterija. Racionalno i odgovorno propisivanje antibiotika preduvjet su za njihovu efikasnost u borbi s bakterijama. Antibiotski pritisak osobito je izražen u bolničkom sustavu, koji je pogodan i za širenje različitih multirezistentnih bakterija.

Sedam godina praćenja bolničke potrošnje antibiotika omogućuje analizu potrošnje u svakoj bolnici, osobito praćenje trendova potrošnje kako ukupne, tako i po klasama. Usporedo s tim, praćenje kretanja bakterijske rezistencije je dobar indikator provođenja bolničke higijene i potrošnje antibiotika. Bolnička potrošnja antibiotika na razini države lagano opada, ali još uvijek neke bolnice pokazuju iznimno visoku potrošnju ili čak trend porasta potrošnje. Kako bi ostvarili racionalno propisivanje antibiotika, u narednim godinama, potrebno je veliko zalaganje različitih profila stručnjaka.

Antibiotic consumption in Croatian Hospitals

As in the previous years, in 2013, the data on hospital antibiotic consumption of class J01A antibiotics was obtained in accordance with the ATC classification. The data was collected in packages/ampoules and entered in the ABC calculator which was adjusted to the Croatian market.

To express the antibiotic consumption, it was necessary to obtain the administrative data collected from each hospital on a separate form (the number of bed days, number of admissions, number of beds, number of children's beds, and number of ICU's).

In the past three years the data from day care was also obtained (the number of therapeutic days), which allows a more objective view of the antibiotic consumption in relation to the hospital activity.

Until 2006 the antibiotic consumption was monitored based only on the wholesales data. Monitoring of hospital antibiotic consumption based on another source, i.e. the data obtained from hospital pharmacies, began with the foundation of the Interdisciplinary Section for Antibiotic Resistance Control (ISKRA) in 2006.

Collecting the data on hospital antibiotic consumption from hospital pharmacies enabled us to calculate the consumption of antibiotics and to express it in defined daily doses (DDD) per 100 bed days (BD), which is a more precise indicator of consumption in relation to the consumption expressed per thousand inhabitants per day (TID), as was the practice until 2006.

Through years of parallel monitoring of antibiotic consumption from two sources (hospital pharmacies and the wholesales data) the differences in total consumption and in different antibiotic classes were detected (Table 5, Figure 4). The same was noticed in 2013 both in total consumption and in consumption of different classes of antibiotics. The greatest divergence, in favour of higher spending according to the data obtained in the hospital pharmacies, was detected in consumption of penicillins (J01C), followed by aminoglycosides (J01G), other groups of antibiotics (J01X) and macrolides (J01F). According to the data obtained from the wholesalers, higher spending was observed in class J01D and in class of macrolides-lincozamides-streptogramin (J01F). The reasons for these differences are not entirely clear.

In 2014 there was a possibility of sending the hospital consumption data by e-mail on the following address: iskra.antibiotici@gmail.com, along with the previous practice of sending the data via post on a CD to the Ministry of Health. The reason for that is the simplicity and less time consuming process of collection, analysis and verification of data. There is already a regular up and running practice of sending the processed data for a verification in each institution with a possibility of comparison of monitored consumption in all the previous years for the respective hospital.

Parallel monitoring of antibiotic consumption from the two sources (wholesales and hospital pharmacies) tracks the difference in consumption, which in 2013 amounted to 0.09 TID, the smallest amount recorded to date (Table 5, Figure 4).

Compared to the previous year, according to the data obtained from hospital pharmacies, hospital consumption decreased (1,8 DDD/TID) (Table 5, Figure 4), or 20.10 DDD/100 BD if hospital bed days were used as a denominator (Table 6, Figure 5). The fall in consumption

can be observed in all classes except quinolones (J01M), which showed an increase (from 4,66 to 5,00 DDD/100 BOD) together with class J01X (other antibiotics) from 2,82 to 3,05 DDD/100 BOD (Table 7, Figure 6).

In the past three years, consumption of class J01A (tetracyclines) decreased. In addition to that, class J01E (sulphonamides), which is the least represented class in the antibiotic usage, also shows a downward trend.

Classes J01C (penicillins) and J01D (cephalosporins) also show a decline in their consumption in the past three years. In 2013 there was a minimal difference in consumption between those two most represented classes (0,73/100 BOD). Their consumption accounts for over 60% of total antibiotic consumption (Table 7, Figure 6).

In class J01F (macrolides, lincozamides, streptogramin) and in class J01G (aminoglycosides) there is a declining trend since 2011.

In 2013 there is a notable consumption growth in quinolones (J01M) and in class J01X, which has for the first time since the beginning of monitoring of antibiotic consumption, exceeded the value of 3 DDD/100 BD (Table 7, Figure 6).

In 2013 the consumption of antibiotics was monitored in 13 **clinical institutions** (Table 8).

Although reorganisation of the hospital system led to merging of several different institutions into one, some institutions continued monitoring as a separate organizational unit because of the domain of the respective institution and because of the possibilities of a better analysis of antibiotic consumption. Clinical Institutions K10 and K12 were merged with larger clinical centres. Through analysis of antibiotic consumption data in 13 clinical institutions we can notice a considerable consumption range (from 18, 4 to 140, 5 DDD/100 BD). Those large differences arise from the different profiles of different clinical institutions and not all of them are comparable. Each hospital, on its own, can track trends in the total consumption, as well as the structure of consumption, which is a useful indicator of the quality of prescribing antibiotics.

Eight clinics (K 01; K 04; K 05; K 06; K 08; K 11; K 13; K 14) noted a decrease in antibiotic consumption in comparison to the year before. Particularly impressive decrease in antibiotic consumption is seen in clinic 6 (K 06), which has, due to the high value, been further examined and verified as a correct data. Stated reason for this is the introduction of special measures and strictly controlled regime of prescribing antibiotics, especially of certain classes which resulted in significant decline in consumption (Figure 7).

In five clinics (K 02; K 03; K 07; K 09; K 15) there is an observed increase in consumption. The greatest leap in the consumption growth was noted in clinic 9 (K 09) (9, 6 DDD/BD), while in others the increase was very discrete. Figure 7 shows trends in antibiotic consumption for each clinical institution.

Antibiotic consumption in a group of **general hospitals** ranges from 24,1 to 72,4 DDD/100 BD, which indicates a significant difference in prescribing antibiotics in the most homogenous group of hospitals (Table 9). One third of general hospitals (7) has a consumption range from 41 to 50 DDD/100 BD. An equal number of hospitals (6) has a consumption range from 51 to 60 DDD/100 BD and from 61 to 70 DDD/BD. Two general hospitals (O 07; O 20) are the greatest consumers among all general hospitals, with a

consumption above 70 DDD/100 BOD (Table 9). Out of 22 general hospitals, 13 of them show a decline in the antibiotic consumption (O 01; O 03; O 04; O 07; O 08; O 12; O 15; O 17; O 18; O 19; O 22; O 23; O 24). Particularly large drop in consumption was recorded in two hospitals O 01 (16,7 DDD/100 BOD) and O 04 (14,4 DDD/100 BOD). Other hospitals show much more discrete decline. Hospital O 02 is in the group of hospitals with consumption range from 41 to 50 DDD/100 BOD and in the past seven years their consumption is within that range, and in the last two years shows almost identical consumption. Like in the previous years, hospital O 21 continues to show an increasing trend in consumption (Figure 8).

Psychiatric hospitals, as institutions with the lowest consumption of antibiotics, have a consumption range from 4,9 to 14,2 DDD/100 BOD (Table 10). In 2013, hospital P 09 is distinguished for its high jump in the antibiotic consumption. This is the youngest hospital by its foundation and the last one to get involved in the monitoring of antibiotic consumption.

The consumption of antibiotics among other psychiatric hospitals is mostly the same, except in hospital P 02 which shows a significant drop in consumption. Psychiatric hospital P 05 has stopped the increasing trend of antibiotic consumption in 2013 (Figure 9).

Specialized hospitals are divided in two large groups regarding their work domain and as such they show a big range in antibiotic consumption. In first group there are 11 hospitals which are intended for treatment (acute/chronical), while in other there are 12 institutions intended for rehabilitation. The first group has the consumption range between 13, 1 and 61, 5 DDD/100 BOD. In the other group the range is between 0, 7 and 14, 2 DDD/100 BOD (Table 11, Figure 10).

Antibiotics are a group of drugs of the outmost significance for modern medicine, so the monitoring of antibiotic consumption is of particular importance. Poorly selected and wrongly applied antibiotic therapy encourages the emerging bacterial resistance. Rational and responsible prescribing of antibiotics are a prerequisite for their effectiveness in fighting the bacteria. The antibiotic pressure is especially expressed in the hospital system which is very suitable for spreading different multiresistant bacteria.

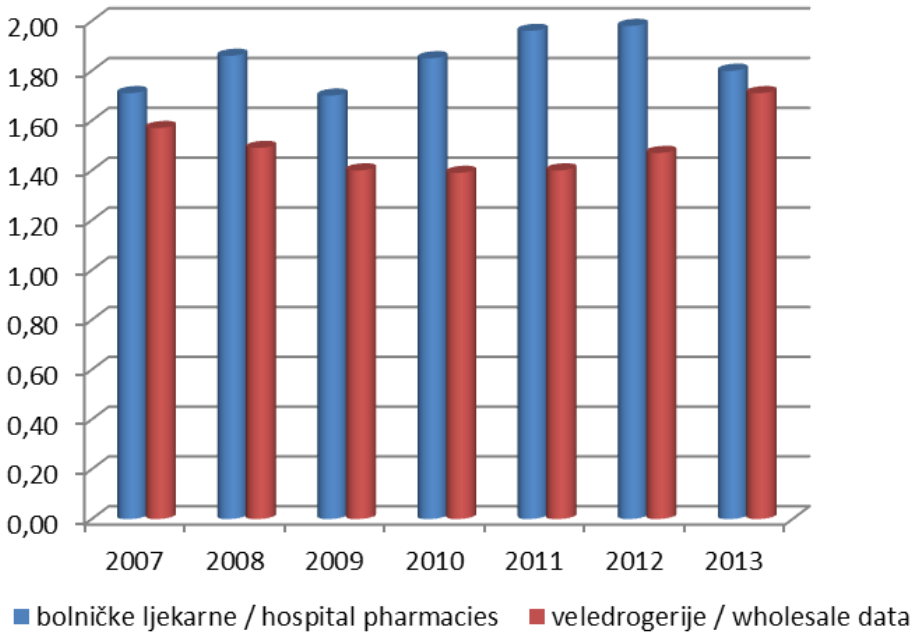
Seven years of monitoring hospital antibiotic consumption gives us the opportunity for consumption analysis in each hospital and for monitoring the consumption trends, both total and in different classes. Along with that, monitoring of bacterial resistance is a good indicator of compliance with hospital hygiene measures and hospital antibiotic use policy.

Hospital antibiotic consumption at the state level is slowly decreasing, but there are still hospitals with a very high rate of consumption or with an increasing consumption trend. In order to achieve rational prescribing of antibiotics in the coming years, a huge commitment of different profiles of experts is required.

Tablica-*Table 5.*
Bolnička potrošnja antibiotika (DDD/TID) usporedba podataka bolničkih ljekarni i veledrogerija
Hospital antibiotic consumption (DDD/TID) comparison between hospital pharmacy data and wholesales data

godina year	bolničke ljekarne hospital pharmacies	veledrogerije wholesales data
2007	1,71	1,57
2008	1,86	1,49
2009	1,70	1,40
2010	1,85	1,39
2011	1,96	1,40
2012	1,98	1,47
2013	1,80	1,71

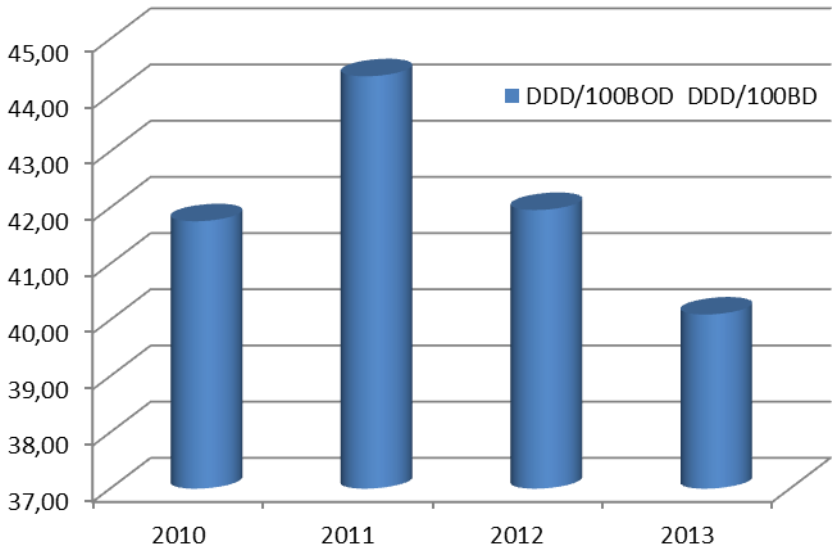
Slika-*Figure 4.*
Bolnička potrošnja antibiotika (DDD/TID) usporedba podataka bolničkih ljekarni i veledrogerija
Hospital antibiotic consumption (DDD/TID) comparison between hospital pharmacy data and wholesales data



Tablica-*Table 6.*
Bolnička potrošnja antibiotika (DDD/100 BOD)
H*ospital antibiotic consumption (DDD/100 BD)*

godina **year**	**DDD/100 BOD** **DDD/100 BD**
2010	41,76
2011	44,34
2012	41,96
2013	40,10

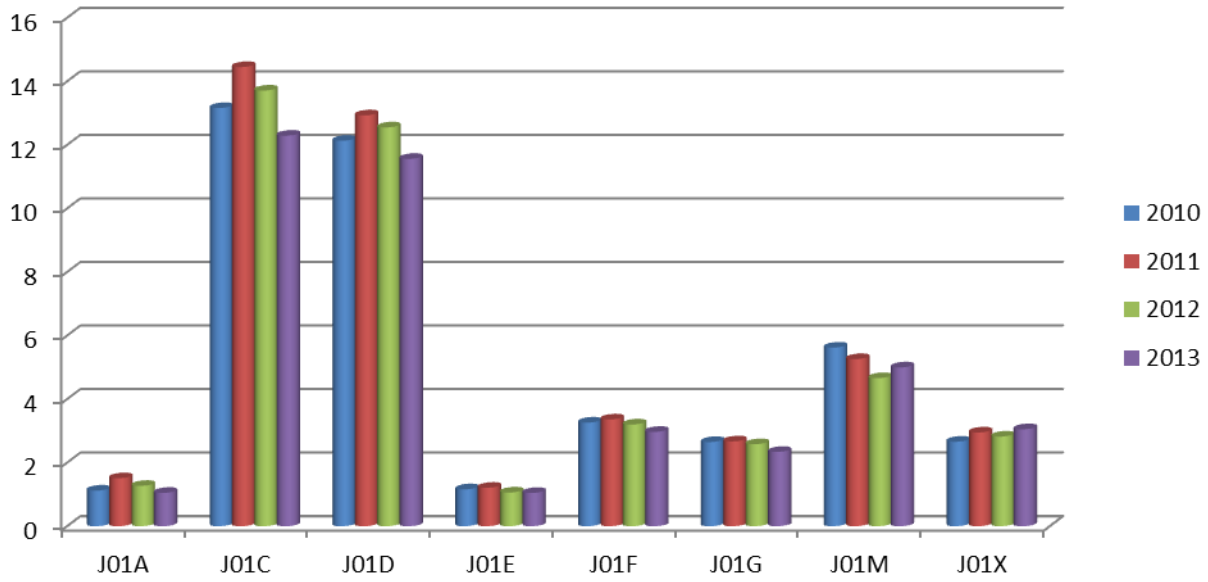
Slika-*Figure 5.*
Bolnička potrošnja antibiotika (DDD/100BOD)
H*ospital antibiotic consumption (DDD/100 BD)*



Tablica-*Table 7.*
Bolnička potrošnja antibiotika (DDD/100 BOD) po klasama, izvor podataka - bolničke ljekarne
Hospital antibiotic consumption (DDD/100 BD) by class, origin of data - hospital pharmacies

klasa/class	godina/year			
	2010	2011	2012	2013
J01A	1,12	1,51	1,27	1,05
J01C	13,16	14,45	13,71	12,29
J01D	12,13	12,93	12,55	11,56
J01E	1,16	1,21	1,06	1,05
J01F	3,26	3,36	3,2	2,97
J01G	2,65	2,67	2,58	2,34
J01M	5,62	5,26	4,66	5,00
J01X	2,66	2,95	2,82	3,05

Slika-*Figure 6.*
Bolnička potrošnja antibiotika (DDD/100 BOD) po klasama, izvor podataka - bolničke ljekarne
Hospital antibiotic consumption (DDD/100 BD) by class, origin of data - hospital pharmacies

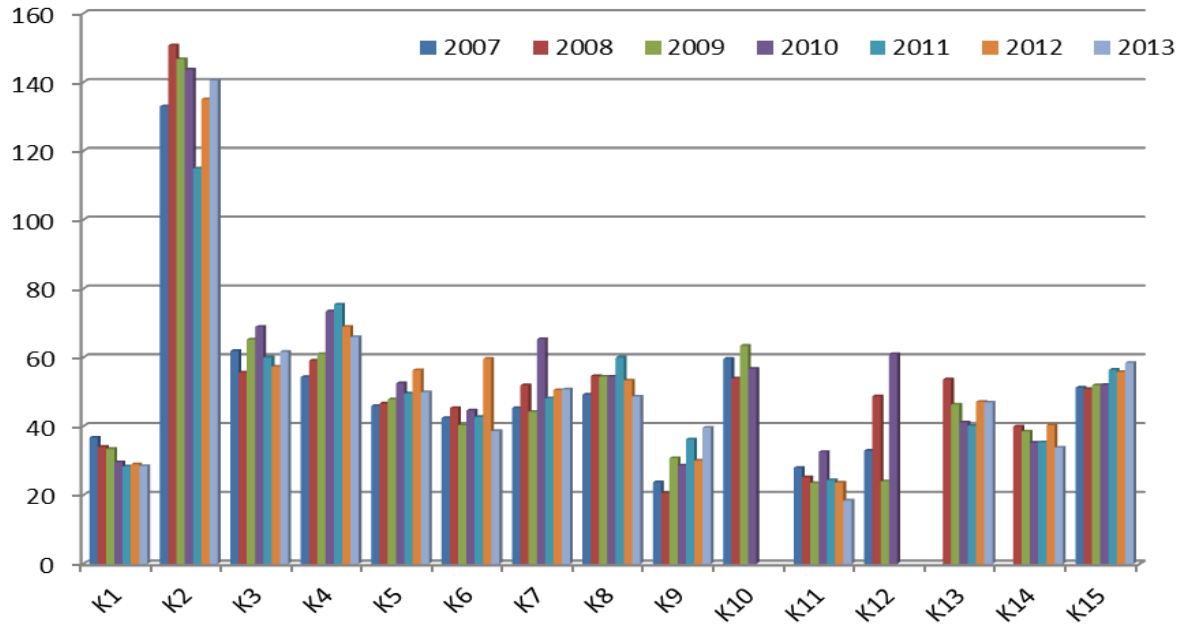


Tablica-*Table 8.*
Kliničke ustanove - potrošnja antibiotika 2013.
Clinical insitutions – antibiotic consumption in 2013

	DDD/100 BOD, DDD/100BD								
USTANOVA INSTITUTION	UKUPNO TOTAL	JO1A	JO1C	JO1D	JO1E	JO1F	JO1G	JO1M	JO1X
K 01	28,4	0,0	7,5	9,7	1,1	4,0	3,7	0,4	2,0
K 02	140,5	3,1	71,9	32,5	2,5	11,1	3,1	8,4	8,0
K 03	61,6	0,3	23,1	15,6	2,7	3,8	2,5	7,7	5,9
K 04	65,9	3,0	24,1	15,3	2,9	3,9	1,9	10,0	4,9
K 05	49,9	1,5	17,4	10,9	0,9	3,8	3,6	8,1	3,8
K 06	38,6	0,6	8,8	16,2	1,2	2,6	2,6	3,1	3,6
K 07	50,7	0,5	13,0	14,4	1,7	4,2	2,3	8,5	6,1
K 08	48,6	1,6	9,9	18,3	1,3	2,3	1,8	8,2	5,1
K 09	39,6	0,0	1,2	32,6	0,2	0,5	1,1	3,5	0,4
K 10*									
K 11	18,4	2,1	5,0	7,6	0,4	0,5	0,5	0,4	1,9
K 12*									
K 13	46,9	0,0	10,7	5,8	2,3	3,3	14,9	6,4	3,4
K 14	33,8	0,2	10,1	15,5	0,9	2,1	1,9	0,8	2,2
K 15	58,4	4,0	22,1	13,5	0,0	5,1	2,6	10,0	4,8

* bolnice koje su ušle u sastav drugih kliničkih ustanova
these hospitals merged in other clinical hospitals

Slika-*Figure 7.*
Kliničke ustanove - potrošnja antibiotika 2007.-2013.
Clinical insitutions – antibiotic consumption in 2007-2013

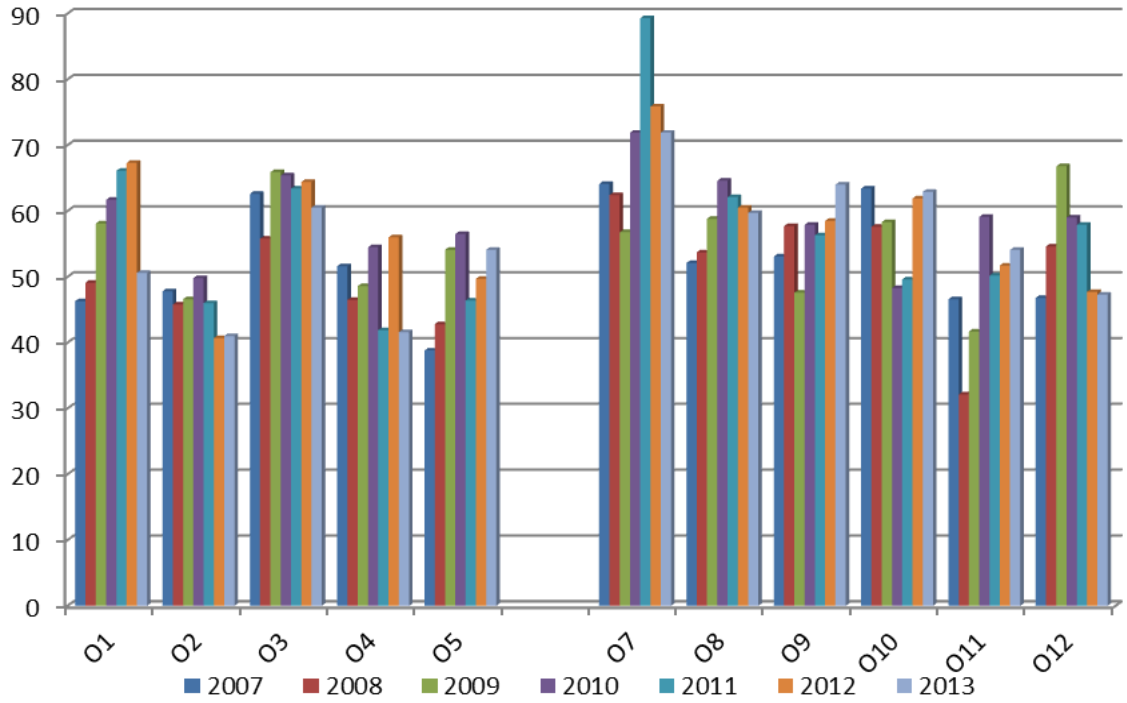


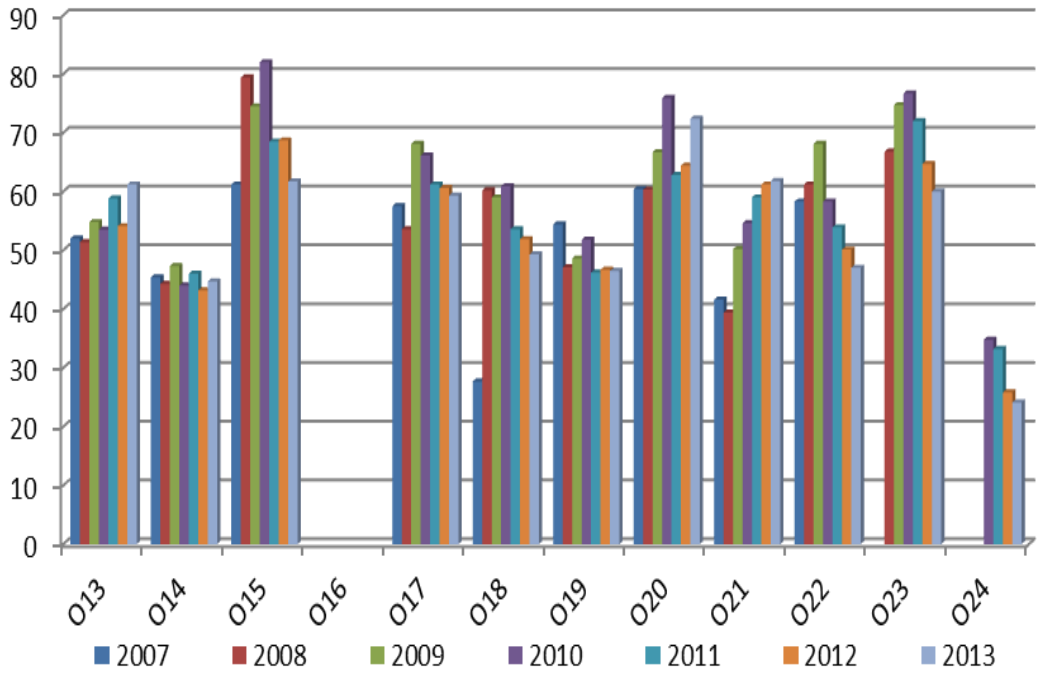
Tablica-*Table 9.*
Opće bolnice - potrošnja antibiotika 2013.
General hospitals – antibiotic consumption in 2013

USTANOVA INSTITUTION	UKUPNO TOTAL	DDD/100 BOD, DDD/100 BD							
		JO1A	JO1C	JO1D	JO1E	JO1F	JO1G	JO1M	JO1X
O 01	50,6	2	17,8	14,8	0,4	3,9	5,2	2,5	4
O 02	41	0,6	22,2	9,3	0,3	1,9	2,4	2,5	2,1
O 03	60,5	4,3	10,4	26,6	0,9	6,2	3,4	3,1	5,6
O 04	41,6	2,6	5,5	14,2	0,8	5,4	5,5	6	1,6
O 05	54,1	3,1	23,8	8,1	0,8	4,1	4,9	5,7	3,6
O 06*									
O 07	71,9	0,7	26,1	19,7	1,7	6,2	9,4	5,5	2,6
O 08	59,7	3,9	22,8	12	2,3	6,1	3	5,9	3,6
O 09	64	0,9	19,3	22,8	0,9	5,1	3,5	7,2	4,4
O 10	62,9	0,8	17,2	24,6	0,6	5,3	3,8	3,9	6,7
O 11	54,1	1,7	19,1	15,9	1,1	4,6	2,7	5,8	3,3
O 12	47,3	1,6	16,7	11,8	0,8	3,8	1,5	8,4	2,7
O 13	61,2	0,5	18,7	24,8	0,6	6,1	2,3	4,4	3,9
O 14	44,7	3,9	18,6	10,3	2,1	2,4	2,6	2,2	2,7
O 15	61,7	3	22,3	18,1	0,4	4,5	5,2	4	4,2
O 16**									
O 17	59,3	1,1	18,4	19,9	0,7	4,1	2,9	7,8	4,3
O 18	49,3	1,8	20,2	11,7	0,7	2,3	2,2	7	3,4
O 19	46,5	0,2	19,3	9,5	1	2,9	3,7	7,1	2,8
O 20	72,4	3,8	12,5	32,2	0,3	5,4	2,8	10,8	4,6
O 21	61,8	0,5	21,7	14,7	0,6	6,4	5,5	6,5	5,9
O 22	47	0,7	13,5	13,1	0,5	3,9	2,8	10	2,5
O 23	60	1,3	21,3	16,2	0,4	6,6	3,8	5,2	5,2
O 24	24,1	0	10,3	3,6	1,5	1,1	2	3,8	1,8

*premještena u skupinu specijalnih bolnica / transferred to the group of specialized hospitals
**premještena u skupinu kliničkih bolnica / transferred to the group of clinical hospitals

Slika-*Figure 8.*
Opće bolnice - potrošnja antibiotika 2007.-2013.
General hospitals – antibiotic consumption 2007-2013

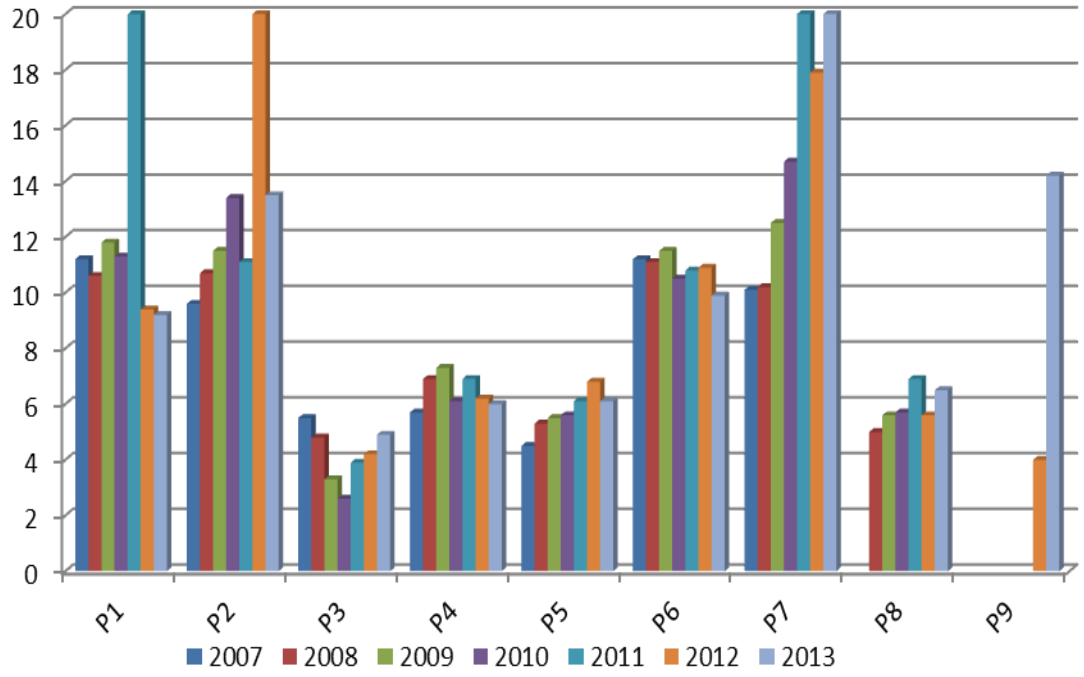




Tablica-*Table 10.*
Psihijatrijske ustanove - potrošnja antibiotika 2013.
Psychiatric institutions – antibiotic consumption in 2013

USTANOVA INSTITUTION	DDD/100 BOD, DDD/100BD								
	UKUPNO TOTAL	JO1A	JO1C	JO1D	JO1E	JO1F	JO1G	JO1M	JO1X
P 01	9,2	0,2	5,1	1	0,7	0,7	0,2	0,7	0,5
P 02	13,5	0,1	6,3	3	0,7	0,8	0,1	2,2	0,4
P 03	4,9	0	2,8	0,9	0	1,3	0	0	0
P 04	6	0,5	3,3	1	0,3	0,3	0	0,5	0,1
P 05	6,1	0,1	3	1,1	0,1	0,9	0	0,9	0
P 06	9,9	0,1	5,4	0,8	0,4	1,3	0,1	1,5	0,2
P 07	23	0,2	2,8	10,9	0,5	0,4	3,9	1,9	2,4
P 08	6,5	0,7	3,2	0,9	0,2	0,2	0,2	1	0,1
P 09	14,2	1	4,8	2,2	0,2	0	0,7	2,1	3,2

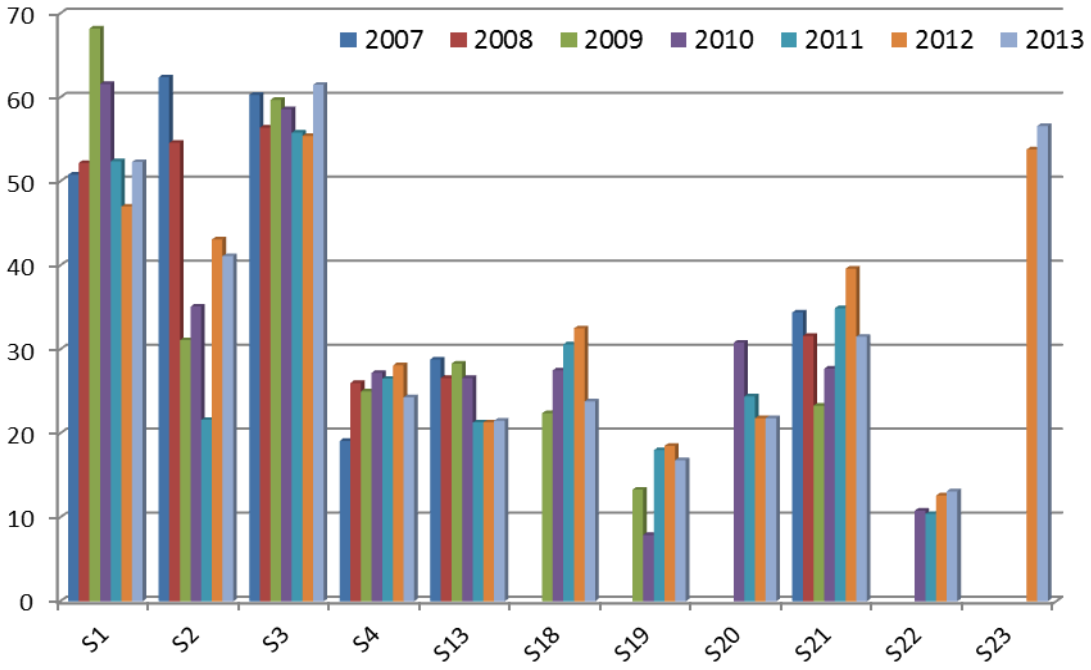
Slika-*Figure 9.*
Psihijatrijske ustanove - potrošnja antibiotika 2007.-2013.
Psychiatric institutions – antibiotic consumption 2007-2013

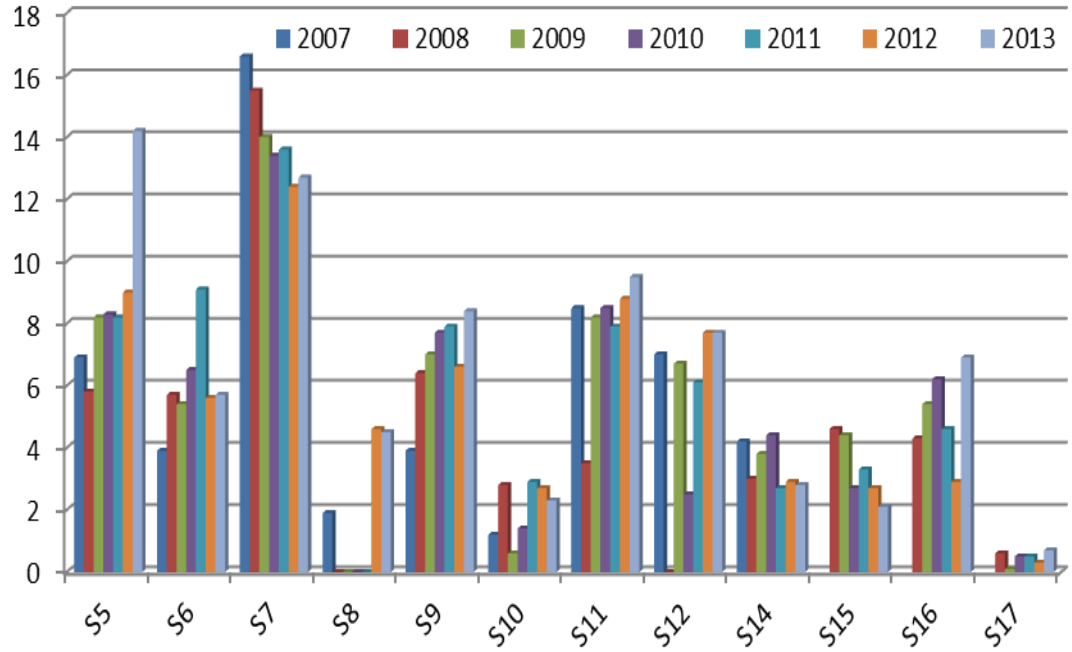


Tablica-*Table 11.*
Specijalne bolnice - potrošnja antibiotika 2013.
Specialised hospitals – antibiotic consumption in 2013

	DDD/100 BOD, DDD/100 BD								
USTANOVA 'IINSTITUTION	UKUPNO TOTAL	JO1A	JO1C	JO1D	JO1E	JO1F	JO1G	JO1M	JO1X
S 01	52,3	1,5	15,3	7,9	1	6,6	5,7	13	1,2
S 02	41,1	0	11,5	20,7	0,2	8,7	0	0	0
S 03	61,5	1,7	20,5	10	2,7	7,2	8,5	10,1	0,9
S 04	24,3	0,9	10,8	2,9	3,8	0,4	1,5	2,7	1,3
S 13	21,5	4	4,3	3,5	1,8	0,8	1,4	2,8	2,8
S 18	23,8	1	11,9	6	0,2	0,2	0,2	3,3	0,9
S 19	16,8	0	3,7	7	2,4	2	0,5	2,7	0,3
S 20	21,8	0	6,4	9,4	0	1,3	0	2,6	2,1
S 21	31,5	0	17	7	0,2	0,9	0,6	4,1	1,5
S 22	13,1	0,2	4	5,1	0	1,7	1,5	0,3	0,2
S 23	56,6	0	0	30,8	0	22,4	1,6	0	1,9
S 05	14,2	0,4	4,6	3,1	0,7	0,6	2,6	2,3	0
S 06	5,7	0	2,2	0,5	0,6	0,1	0,1	1,2	0,9
S 07	12,7	0,1	4	2,8	0,5	0,9	0,7	3	0,7
S 08	4,5	0,1	2,5	0,6	0,1	0,3	0	0,9	0,1
S 09	8,4	0,1	4,8	1,1	0,5	0,5	1,3	0,2	0
S10	2,3	0,2	0,7	0,4	0,4	0,1	0	0,5	0,1
S11	9,5	0,2	3,7	2,4	0,9	0,5	0,2	1,3	0,3
S12	7,7	0,6	4,4	0,3	0	1,6	0	0,8	0
S14	2,8	0	1	1,1	0,4	0,3	0	0	0
S15	2,1	0	0,4	1,1	0	0,5	0	0	0,1
S16	6,9	0,4	3,4	2	0,2	0,4	0	0,4	0,2
S17	0,7	0	0,5	0,1	0,1	0	0	0	0

Slika-*Figure 10*
Specijalne bolnice - potrošnja antibiotika 2007.-2013.
Specialised hospitals – antibiotic consumption 2007-2013





ATK KLASIFIKACIJA ANTIBIOTIKA:
ATC CLASSIFICATION OF ANTIBIOTICS

J01A – TETRACIKLINI / *TETRACYCLINES*
J01B – AMFENIKOLI / *AMPHENICOLS*
J01C – ß LAKTAMI – PENICILINI / *ß LACTAM-PENICILLINS*
J01D – ß LAKTAMI – CEFALOSPORINI / *ß LACTAM-CEPHALOSPORINS*
J01E – SULFONAMIDI I TRIMETOPRIM / *SULFONAMIDES AND TRIMETHROPIM*
J01F – MAKROLIDI, LINKOZAMIDI I STREPTOGRAMIN / *MACROLIDES, LINCOZAMIDES AND STREPTOGRAMIN*
J01G – AMINOGLIKOZIDI / *AMINOGLYCOSIDES*
J01M – KINOLONI /*QUINOLONES*
J01X – OSTALI (GLIKOPEPTIDI, POLIMIKSIN, METRONIDAZOL, NITROFURANTOIN) / *OTHERS (GLYCOPEPTIDES, POLYMYXIN, METRONIDASOLE, NITROFURANTOIN*

POGLAVLJE/CHAPTER 5.

VANJSKA KONTROLA KVALITETE, 2013.
EXTERNAL QUALITY CONTROL, 2013

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Reference Centre for Antibiotic Resistance Surveillance of the Croatian Ministry of Health

Vanjska kontrola kvalitete
External Quality Control

Opis sojeva za kontrolu: <u>proljeće 2013</u>

Soj 01 / 13 ***Streptococcus pneumoniae*** je soj ATTC 49619. Soj je smanjeno osjetljiv (I) na penicilin, osjetljiv na ampicilin i ostale antibiotike. Vrijednost oksacilina disk difuzijom je u rasponu od 8 - 14 mm. MIK za penicilin je od 0,25 - 1 µg/mL, a za ampicilin od 0,06 - 0,25 µg/mL. Interpretacija za penicilin ovisila je o kliničkoj dijagnozi. Prema EUCAST smjernicama u slučaju pneumonije klinička intepretacija je ovisila o vrijednosti MIK-a za penicilin. U slučaju meningitisa soj je rezistentan na penicilin (> 0,064 µg/mL). Osjetljivost na kinolone testirana upotrebom norfloksacin diska od 10 µg kretala se u rasponu od 18 - 24 mm.

Hrvatski su laboratoriji za oksacilin dobilii vrijednosti u rasponu od 6-20 mm (slika 1). Vrijednost MIKa za penicilin odredili su u rasponu od 0,047 - 0,5 µg/mL (slika 2). Dva hrvatska laboratorija od 34 su imala MIK ≤ 0,064 µg/mL te su proglasili soj osjetljivim na penicilin. Dva su laboratorija određivala osjetljivost samo disk difuzijom i za penicilin i za ampicilin. MIK za ampicilin se kretao u rasponu od 0,064 - 0.25 µg/mL (slika 3). Norfloksacin disk u određivanju osjetljivosti kinolona koristilo je 33/34 laboratorija i svi laboratoriji su točno utvrdili da je soj osjetljiv na kinolone (slika 4). Raspon vrijednosti naših laboratorija kretao se od 13 - 25 mm. Od 34 laboratorija 19 ih je interpretiralo penicilin kao samnjeno osjetljiv (I). Prema EUCAST smjernicama ti laboratoriji bi trebali za kliničara stavili napomenu o odnosu MIK-a i doze penicilina ako je dijagnoza pneumonija. Soj bi poslalo u referentni centar 31/34 laboratorija (slika 5).

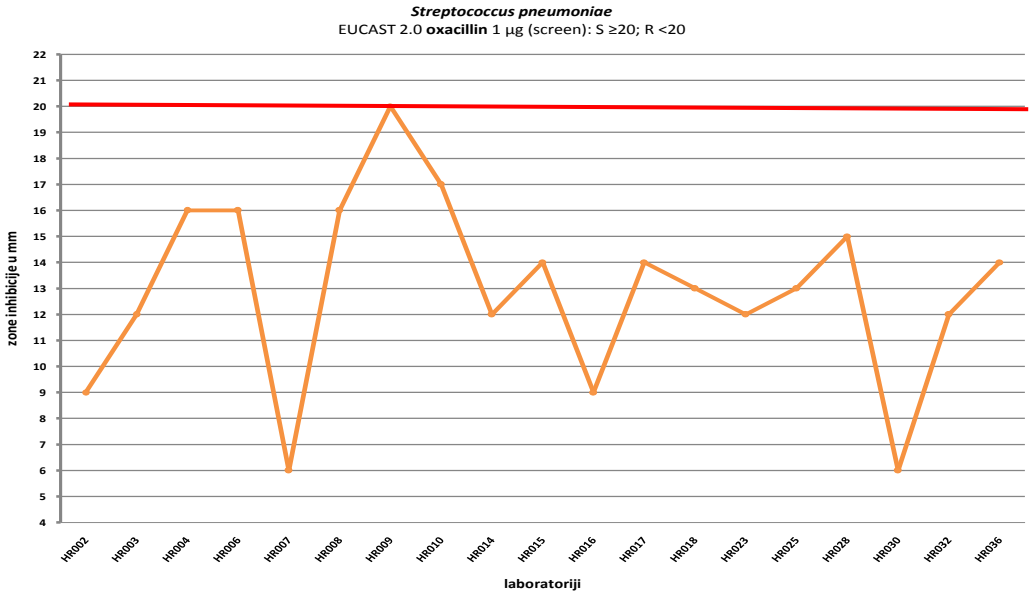
Soj 02 / 13 ***Staphylococcus aureus*** je **MRSA** s dokazanom heterorezistencijom na vankomicin, Mu3 hVISA soj. Soj je osjetljiv na vankomicin, MIK 2 µg/mL.

Vrijednost cefoksitina koju su hrvatski laboratoriji dobili disk difuzijom kretala se od 6 do 9 µg/mL (slika 6). MIK za vankomicin, koju su testirali hrvatski laboratorije, kretao se od 0,75 - 3 µg/mL (slika 7), a teikoplanina od 2-12 µg/mL (slika 8). Od 7 laboratorija koji su soj testirali na heterorezistenciju makro E-testom 5 je točno utvrdilo da se radi o hVISA. Dva su laboratorija koristila GRD test i oba su točno tim testom utvrdila da se radi o hVISA (Tablica 1). Od 34 laboratorija 22 bi poslala soj u referentni laboratorij.

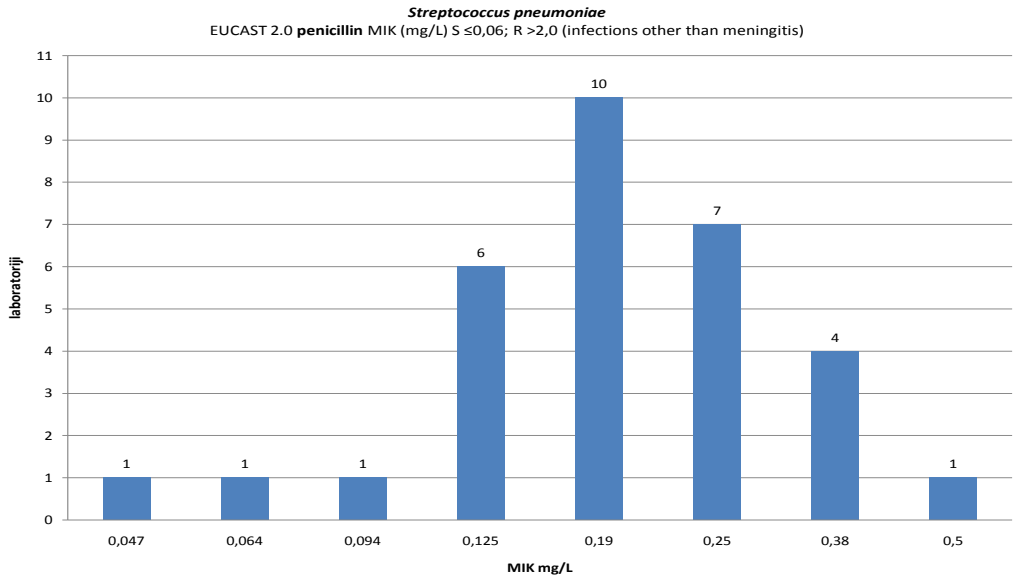
Challenge strains: spring 2013

Strain 01 / 13 ***Streptococcus pneumoniae*** was j ATTC 49619 strain. It was intermediary susceptible to penicillin, but susceptible to ampicillin and other tested antibiotics. Range for oxacillin by disk diffusion was 8 - 14 mm. MIC for penicillin was 0,25 - 1 µg/mL, and for ampicillin 0,06 - 0,25 µg/mL. Interpretation for penicillin was dependent of clinical diagnosis. According EUCAST recommendations for pneumonia dosing was dependent of penicillin MIC. For meningitis strain was resistant to penicillin (> 0,064 µg/mL). Quinolone resistance was screened by norfloxacin disk (10 µg) and range was 18 - 24 mm.
Range for oxacillin for Croatian laboratories was 6-20 mm (figure 1). MIC for penicillin was 0,047 - 0,5 µg/mL (figure 2). Two of 34 Croatian laboratories reported penicillin as susceptible having MIC ≤ 0,064 µg/mL .Two laboratories used only disk diffusion for penicillin and ampicillin. Range for ampicillin MIC was 0,064 - 0.25 µg/mL (figure 3). Norfloxacin disk for screening of quinolone susceptibility used 33/34 laboratories. All laboratories referred correctly that the strain is susceptible to quinolones (figure 4). They reported range of norfloxacin from 13 - 25 mm. Intermediary susceptibility to penicillin reported 19/ 34 Croatian laboratories. For pneumonia those laboratories according EUCAST recommendation those laboratories would report dose of penicillin in dependence to MIC value. Strain would send to reference laboratory 31/34 laboratories (figure 5).
Strain 02 / 13 ***Staphylococcus aureus*** was **MRSA,** heteroresistant to vancomycin**,** Mu3 hVISA strain. MIC for vancomycin was 2 µg/mL (susceptible).
Cefoxitin values of Croatian laboratories by disc diffusion were in range 6 do 9 µg/mL (figure 6). MIC for vancomycin was in range 0,75 - 3 µg/mL (figure 7), and MIC for teicoplanin 2-12 µg/mL (figure 8). Seven laboratories tested strain for heteroresistance to vancomycin by macro E-test and 5 of them correctly reported hVISA. Two laboratories used commercial GRD test and correctly detected hVISA (Table 1). Strain would send to reference laboratory 22/ 34 laboratories.

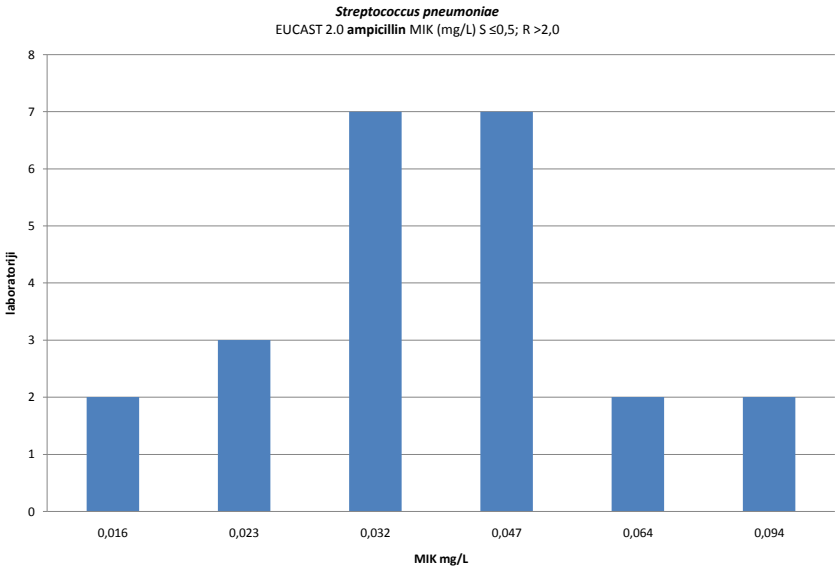
Slika-*Figure 1.*
Soj 01/13 ***S.pneumoniae*** – oksacilin , diskom difuzija
Strain 01/13 ***S.pneumoniae*** – *oxacillin, disk diffusion*



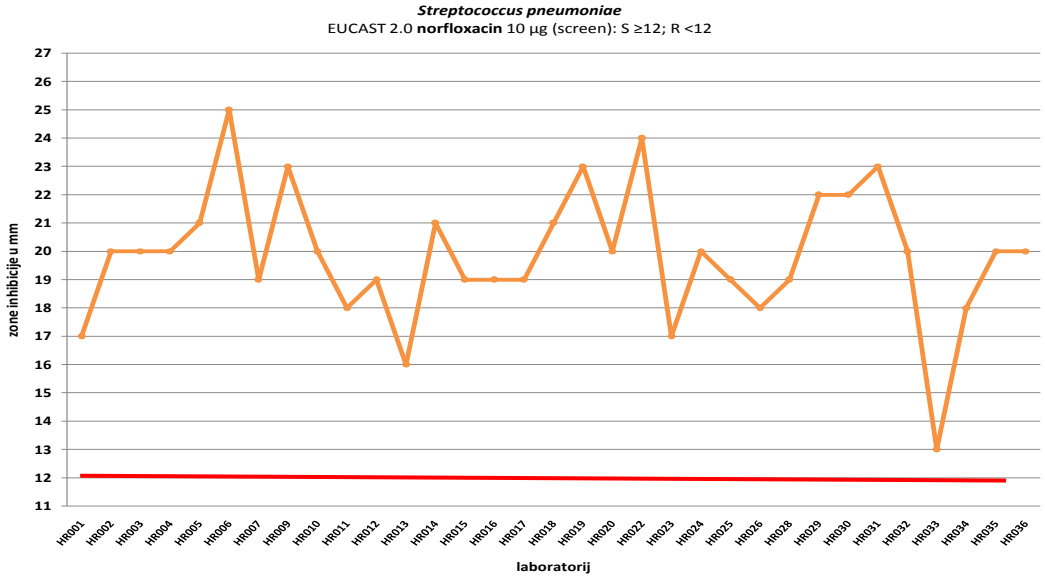
Slika-*Figure 2.*
Soj 01/13 ***S.pneumoniae*** – MIK penicilin
Strain 01/13 ***S.pneumoniae*** – MIC penicillin



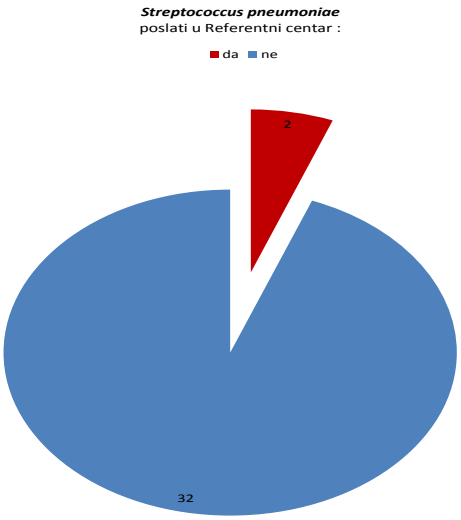
Slika-*Figure 3.*
Soj 01/13 ***S.pneumoniae*** – MIK ampicilin
Strain 01/13 ***S.pneumoniae*** – MIC ampicillin



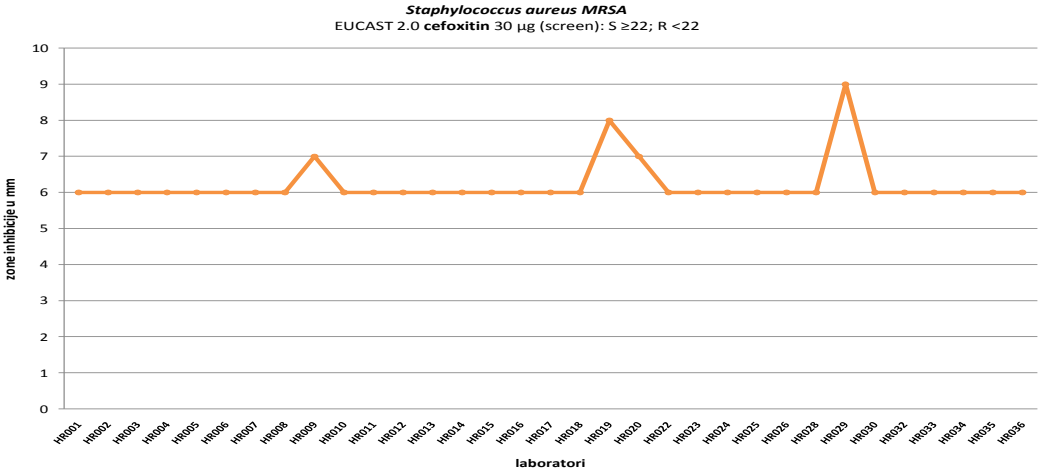
Slika-*Figure 4.*
Soj 01/13 ***S.pneumoniae*** – norfloksacin screening
Strain 01/13 ***S.pneumoniae*** – norfloxacin screening



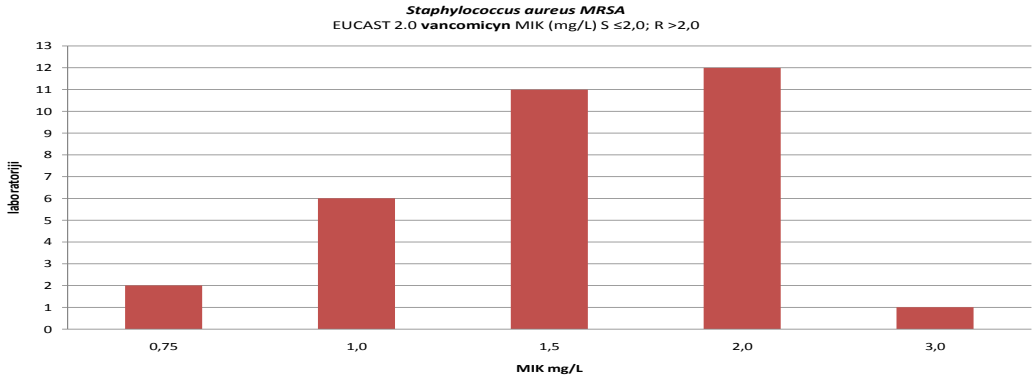
Slika-*Figure 5.*
Soj 01/13 ***S.pneumoniae*** – upućivanje u referentni laboratorij
Strain 01/13 ***S.pneumoniae*** – reference laboratory addmition



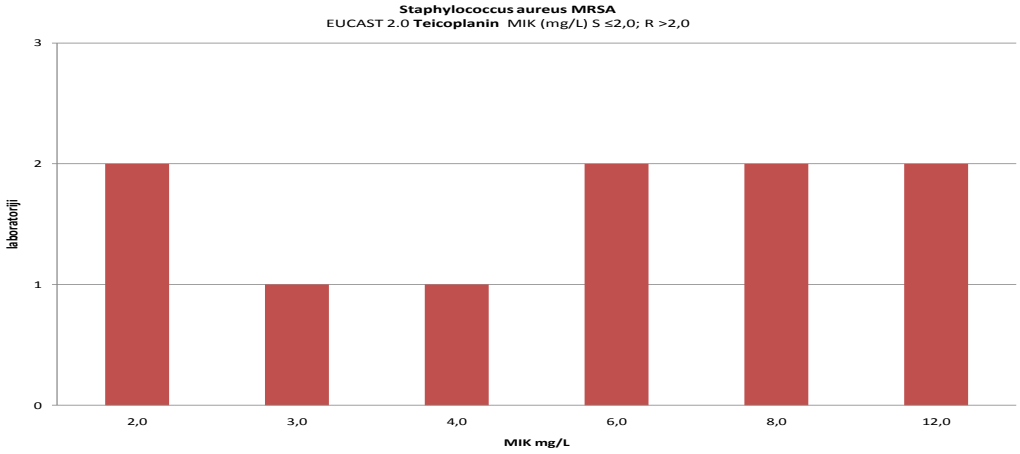
Slika - *Figure 6.*
Soj 02/13 ***S.aureus* MRSA** – testiranje cefoksitin, disk difuzija
Strain 02/13 ***S.aureus* MRSA** – cefoxitin, disk diffusion



Slika-*Figure 7.*
Soj 02/13 ***S.aureus* MRSA** – MIK vancomicin
Strain 02/13 ***S.aureus* MRSA** – MIC vancomycin



Slika-*Figure 8.*
Soj 02/13 ***S.aureus* MRSA** – MIK teikoplanin
Strain 02/13 ***S.aureus* MRSA** – MIC teicoplanin



Tablica-*Table 1*.
Soj 02/13 ***S.aureus* MRSA** – makro E-testi i GRD
Strain 02/13 ***S.aureus* MRSA** – macro E-testi and GRD

	Makro E-testovi		Vitek	GRD	
	VA	TEIKO		VA	TEIKO
HR002	4,0	8,0		1,5	>32,0
HR005	1,5		Hetero VISA, MLS_B		
HR007					
HR008			VA 2,0; TEIKO 4,0		
HR010	3,0	16,0			
HR012	4,0	12,0			
HR015	4,0	16,0			
HR017	4,0	12,0			
HR030			TEIKO 8,0		
HR032	16,0	24,0			
HR033				2,0	>32,0

Zbirni prikaz rezultata za kontrolu: PROLJEĆE 2013.

Lab.	01 / 13 *S.pneumoniae*			02 / 13 *S.aureus* MRSA			
	ID	ATB osim beta laktama	PENICILLIN INTERPRETACIJA	ID	MRSA	ATB	POSLATI U RC
1	+	+	+	+	+	+	DA/Y
2	+	+	+	+	+	+	NE/N
3	+	+	+	+	+	-**	DA/Y
4	+	+	+	+	+	+	NE/N
5	+	+	-*	+	+	+	NE/N
6	+	-	- *	+	+	+	NE/N
7	+	+	-*	+	+	+	DA/Y
8	+	+	+	+	+	+	DA/Y
9	+	+	-*	+	+	+	DA/Y
10	+	+	+	+	+	+	DA/Y
11	+	+	+	+	+	+	DA/Y
12	+	+	+	+	+	+	DA/Y
13	+	-	-*	+	+	+	DA/Y
14	+	+	-*	+	+	+	DA/Y
15	+	+	-*	+	+	+	DA/Y
16	+	-	+	+	+	+	NE/N
17	+	+	-*	+	+	+	DA/Y
18	+	+	+	+	+	+	DA/Y
19	+	+	-*	+	+	+	DA/Y
20	+	+	+	+	+	+	NE/N
21	+	+	+	+	+	+	NE/N
22	+	+	+	+	+	+	NE/N
23	+	+	+	+	+	+	NE/N
24	+	+	+	+	+	+	NE/N
25	+	+	-*	+	+	+	NE/N
26	+	+	+	+	+	+	DA/Y
27	+	+	-*	+	+	+	DA/Y
28	+	+	-*	+	+	+	DA/Y
29	+	+	-*	+	+	+	DA/Y
30	+	+	+	+	+	+	DA/Y
31	+	+	+	+	+	+	DA/Y
32	+	+	-*	+	+	+	DA/Y
33	+	+	-*	+	+	+	NE/N
34	+	+	-*	+	+	+	DA/Y

* manja greška/minor ** velika greška/major *** vrlo velika greška/very major **** greška u interpretaciji/ interpretation error
N= no; Y=yes

Opis sojeva za kontrolu: <u>jesen 2013</u>

Kao jesenja kontrola testiranja osjetljivosti na antibiotike obrađeni su podaci za sojeve koji su testirani u okviru EARS-Net projekta, UK National External Quality Assesment Service for Microbiology (UK NEQAS), distribucija 3228 od 4. studenog 2013. godine. Rezultati su interpretirani prema EUCAST verziji 3.1. od 11.02.2013, s obzirom da su to bili dogovoreni i službeni standardi za Hrvatsku u 2013. godini.

Soj 01/13 (1445): ***Acinetobacter baumannii****:* soj je bio osjetljiv na sve testirane antibiotike. Od 33 hrvatska laboratorija 28 je točno identificiralo soj, a osjetljivost na antibiotike su svi točno odredili (slika1 i 2).

Soj 02/13 (1446): ***Escherichia coli****:* soj je bio rezistentan samo na ampicilin/amoksicilin i osjetljiv na sve ostale testirane antibiotike. Soj je bio granično osjetljiv na amoksicilin s klavulanskom kiselinomj (MIK 8 μg/mL) i 87,4% europskih laboratorija je točno odredilo osjetljivost ovog soja. Laboratoriji koji su koristili disk difuziju su češće pogrešno odredli smanjenu osjetljivost na amoksicilin s klavulanskom kiselinom (14,7% EUCAST, odnosno 18,3% po CLSI).

Samo jedan hrvatski laboratorij je utvrdio da je soj smanjeno osjetljiv na a amoksicilin s klavulanskom kiselinom (slika3).

Soj 03/13 (1447): ***Klebsiella pneumoniae****:* soj producira karbapenemazu OXA-48. Izolati koji proizvode OXA-48 enzime često pokazuju graničnu rezistenciju na karbapeneme i mogu biti osjetljivi na cefalosporine. Soj je bio rezistentan na ertapenem (MIK 8 - 64 μg/mL) i intermedijarno osjetljiv na imipenem i meropenem (MIK 4 μg/mL za oba) po EUCAST interpretaciji odnosno razistentan po CLSI interpretaciji. Većina europskih laboratorija je točno odredilo osjetljivost za ertapenem (98,6%). Za imipenem je točno odredilo osjetljivost 70% laboratorija (35% kao smanjeno osjetljiv i 35% kao rezistentan), a za meropenem 68% laboratorija (35% kao osjetljiv i 33% kao smanjeno osjetljiv). Soj je bio granično osjetljiv na ceftazidim (MIK 1 μg/mL) i 91% europskih laboratorija je točno odredilo da je soj osjetljiv. Obzirom je fenotipski teško utvrditi OXA-48 i njemu slične enzime potrebno je koristiti genotipizaciju.

Svi hrvatski laboratoriji su točno zabilježili rezistenciju na ertapenem, ali ne na imipenem i meropenem (slika 4 i 5). Od 33 laboratorija 14 je imalo poptuno točan antibiogram. Osam labaoratorija je utvrdilo smanjenu osjetljivost na ceftazidim (slika 6).

Soj 04/13 (1448): ***Staphylococcus aureus****:* soj je MRSA rezistentan na klindamicin.
Hrvatski laboratoriji nisu imali problema u identifikaciji i testiranju osjetljivosti ovog soja. Svi laboratoriji (33/33) točno su cefoksitinskim diskom utvrdili rezistenciju ovog soja. Laboratoriji su određivali i MIK za vankomicin koji se za ovaj soj kretao u rasponu od 0,25 do čak 1,5 μg/mL (slika 7), dok se za teikoplanin kretao od od 0,19 do 1,5 μg/mL (slika 8).
Soj 05/13 (1449): ***Streptococcus pneumoniae****:* soj je multirezistentan i osjetljiv je samo na levofloksacin i moksifloksacin. MIK za penicilin je 4-8 μg/mL. Oksacilinski disk se pokazao kao odličan za screening rezistencije ovog soja na penicilin i 99% europskih laboratorija je na taj način točno utvrdilo penicilinsku rezistenciju. Soj je rezistentan i na ceftriakson. Čak 92% europskih laboratorija je ceftrakson označila rezistentnim, ako se radilo o dijagnozi meningitisa, odnosno 84%, ako je dijagnoza bila pneumonija.
Većina hrvatskih laboratorija je utvrdila smanjenu osjetljivost soja na penicili, 23/33 s najčešćom vrijednošću MIK ≥ 3 μg/mL (slika 9), a 21/33 laboratorija su utvrdila rezistenciju na ceftriakson, MIK ≥ 3 μg/mL (slika 10). U kliničkoj interpretaciji 23 laboratorija su soj označila rezistentnim u slučaju pneumonije, 3 intermedijarnim i 6 osjetljivim (tablica 2). Također 22 laboratorija su soj označila rezistentnim na ceftraikson za pneumoniju , 7 intermedijarnim i 2 laboratorija su soj označila osjetljivim za pneumoniju (tablica 2). U kliničkoj interpretaciji penicilina za meningitis nije bilo problem, ali za ceftriakson je 6 laboratorija označilo soj intermedijarno osjetljivim na ceftriakson i 1 laboratorij osjetljivim na ceftriaksona, a 23 labaoratorija rezistentnim (tablica 1).
Soj 06/13 (1450): ***Pseudomonas aeruginosa****:* soj je rezistentan na karbapeneme i sve referentne antibiotike, a osjetljiv na kolistin i granično osjetljiv na piperacilin-tazobaktam (MIK 16 μg/mL). Karbapenemska rezistencija se temeljila na efluksu i gubitku oprD porina, a soj je producirao i VEB ESBL.
Samo su 2/33 hrvatska laboratorija piperacilin tazobaktam označili rezistentnim (slika 11).

Challenge strains: autumn 2013

Quality control of antibiotic susceptibility testing was done using the data for strains tested in EARS-Net project, UK National External Quality Assessment Service for Microbiology (UK NEQAS), distribution 3228 November 4th 2013. Results were interpreted according EUCAST version 3.1. February 11th2013, as agreed and official standards for Croatia in 2013.

Strain 01/13 (1445): ***Acinetobacter baumannii****:* organism was susceptible to all reference agents tested.

Croatian laboratories did not have problems with susceptibility testing, but 28/33 laboratories correctly identified organism (figure1 and 2).

Strain 02/13 (1446): ***Escherichia coli****:* resistant to ampicillin/amoxicillin and susceptible to all other reference antibiotics tested. Susceptibility to amoxicillin-clavulanic acid was borderline (MIC 8 μg/mL) and 87,4% European laboratories correctly reported susceptibility of the strain. Participants using disk diffusion method commonly reported incorrectly strain as intermediate susceptible or resistant to amoxcillin-clavulanic acid (14,7% EUCAST, 18,3% CLSI).

Only one of 33 Croatian laboratories incorrectly reported strain as intermediate susceptible to amoxicillin- clavulanic acid (figure 3).

Strain 03/13 (1447): ***Klebsiella pneumoniae****:* producing OXA-48 carbapenemase. Isolates producing OXA-48 enzymes commonly show borderline resistance to carbapenems and may be susceptible to cephalosporins. Strain was resistant to ertapenem (MIC 8 - 64 μg/mL) and intermediate to both imipenem and meropenem (MIC 4 μg/mL for both), by EUCAST breakpoints but resistant by CLSI. Most European laboratories reported isolate as resistant to ertapenem (98,6%). For imipenem correctly reported 35% laboratories and for meropenem 33%. Strain was borderline susceptible to ceftazidime (MIC 1 μg/mL) and 91% European laboratories correctly reported susceptibility of the strain. Identification of OXA-48 like enzymes by phenotypic method is problem so this has to be confirmed with a genotypic method.

All Croatian laboratories correctly reported ertapenem as resistant, but not to imipenem and meropenem (figure 4 and 5). Only 14/33 laboratories reported all reference antibiotics correctly. Eight laboratories reported intermediate susceptibility to ceftazidime (figure 6).

Strain 04/13 (1448): ***Staphylococcus aureus****:* organism is methicillin resistant and resistant to clindamycin.

Croatian laboratories did not have problems in identification and antibiotic susceptibility testing of this strain. All laboratories reported correctly resistance of the strain by use of cefoxitin disk (33/33). Some laboratories reported MIC for vancomycin (0,25 - 1,5 μg/mL) (figure 7), and for teicoplanin (0,19 - 1,5 μg/mL) (figure 8).

Strain 05/13 (1449): ***Streptococcus pneumoniae****:* multi-resistant strain, susceptible only to levofloxacin and moxifloxacin. MIC for penicillin was 4-8 μg/mL. Oxacillin disk was excellent for screening of the resistance of this strain to penicillin and almost all European laboratories (99%) correctly reported penicillin resistance. Strain was resistant to ceftriaxone too and 92% European laboratories correctly reported resistance to ceftriaxone for pneumonia, but 84% for meningitis.

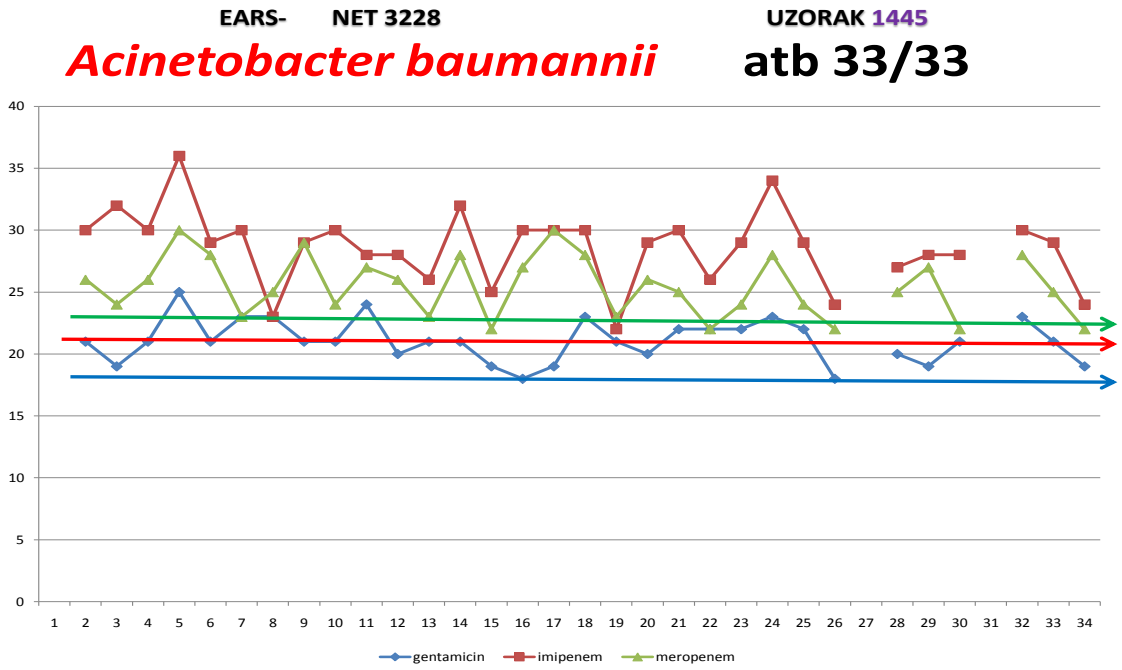
Most of Croatian laboratories (21/33) detected high MIC for penicillin ≥ 3 μg/mL (figure 9 and 10). Clinical interpretation of 23 laboratories for pneumonia was resistant, 3 intermediate and 6 susceptible (table 2). For ceftriaxone 22 laboratories reported as resistant for pneumonia, 7 intermediate and 2 susceptible (table 2). For meningitis there were not problems in interpretation for penicillin, but for ceftriaxone 6 laboratories reported strain as

intermediate, and one laboratory as susceptible while 23 laboratories reported correctly as resistant (table 1).

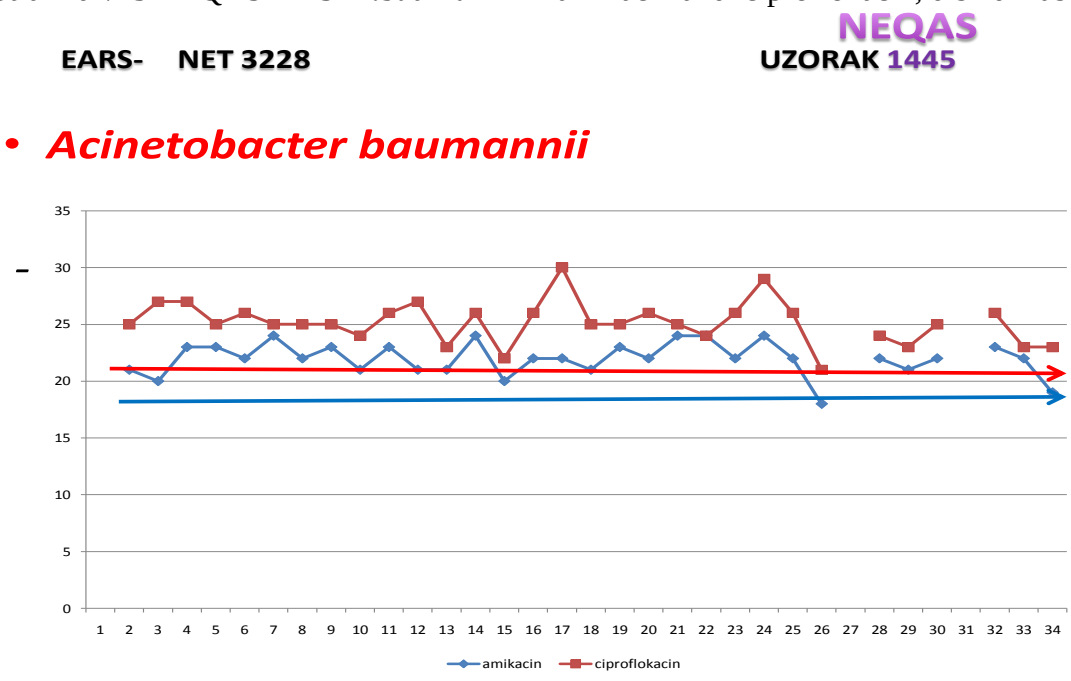
Strain 06/13 (1450): ***Pseudomonas aeruginosa****:* with carbapenem resistance and resistance to all reference antibiotics. It was susceptible to colistin and borderline susceptible to piperacillin-tazobactam (MIK 16 μg/mL). Carbapenem resistance is typical for isolates with upregulated efflux and *oprD* porin loss. At the same time strain produces a VEB ESBL.

Only 2/33 Croatian laboratories incorrectly reported piperacillin- tazobactam as resistant (figure 11).

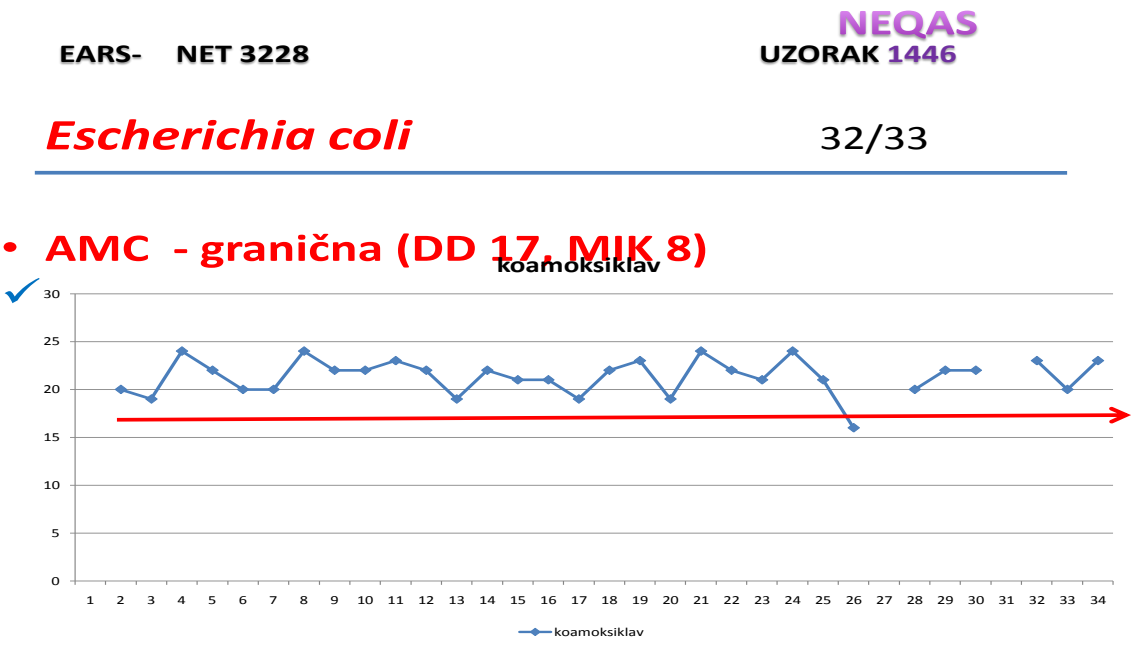
Slika-*Figure 1.*
Soj 01/13 NEQAS 1445 ***A.baumannii*** – gentamicin, imipenem, meropenem, disk difuzija
Strain 01/13 NEQAS 1445 ***A.baumannii*** – gentamicin, imipenem, meropenem, disk diffusion



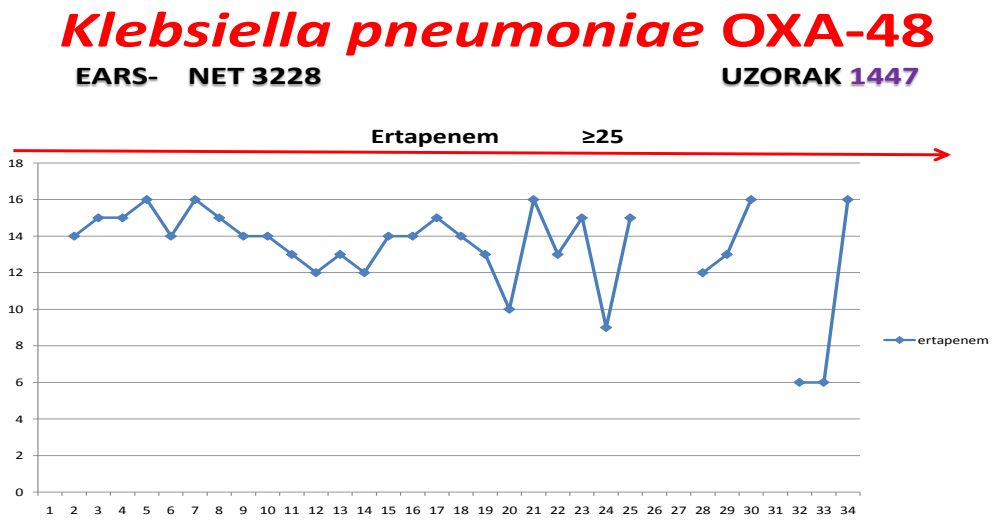
Slika-*Figure 2.*
Soj 01/13 NEQAS 1445 ***A.baumannii*** – amikacin i ciprofloksacin, disk difuzija
Strain 01/13 NEQAS 1445 ***A.baumannii*** – amikacin and ciprofloxacin, disk diffusion



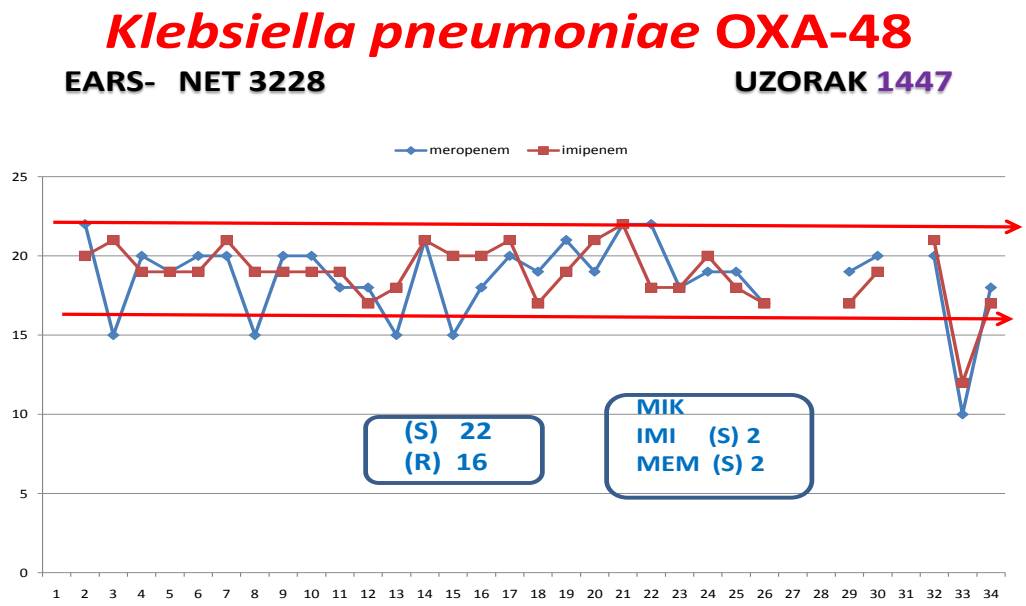
Slika-*Figure 3.*
Soj 02/13 NEQAS 1446 ***E.coli*** – amoksicilin sa klavulanskom ksielinom, disk difuzija
Strain 02/13 NEQAS 1446 ***E.coli*** – amoksicill-clavulanic acid, disk diffusion



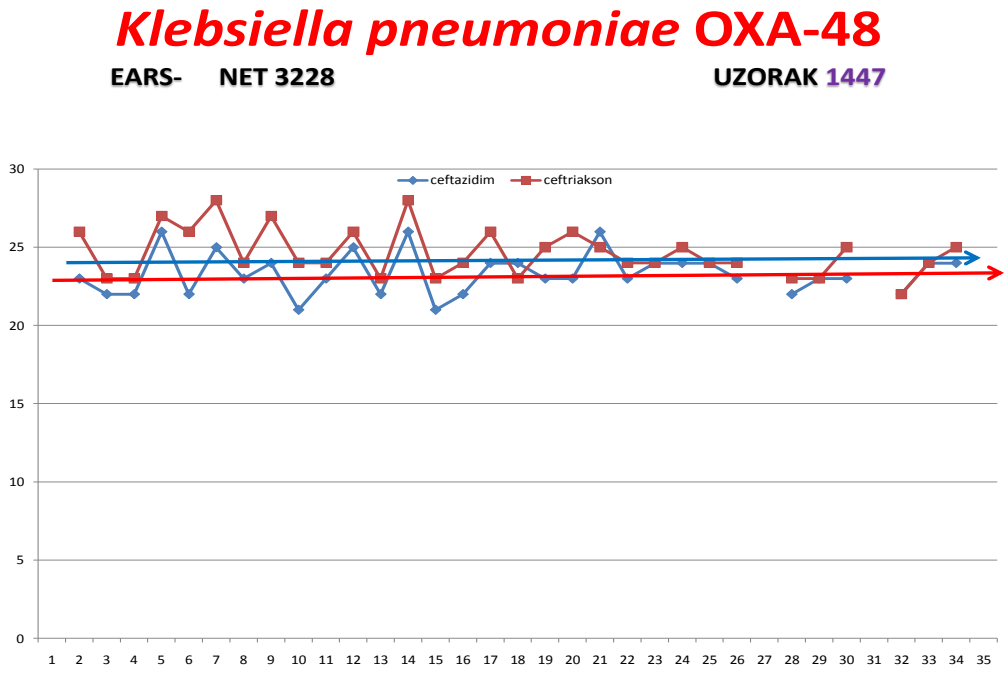
Slika-*Figure 4.*
Soj 03/13 NEQAS 1447 ***K.pneumoniae*** – ertapenem disk difuzija
Strain 03/13 NEQAS 1447 ***K.pneumoniae*** – ertapenem disk diffusion



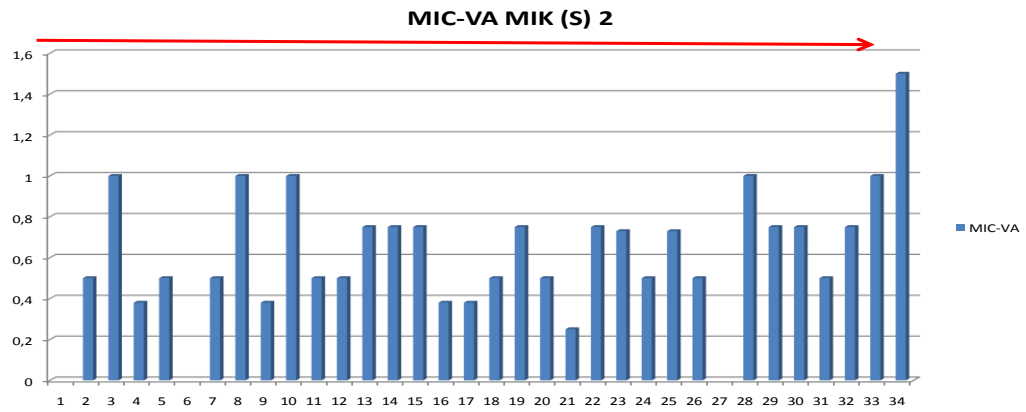
Slika-*Figure 5.*
Soj 03/13 NEQAS 1447 ***K.pneumoniae*** – imipenem i meropenem disk difuzija
Strain 03/13 NEQAS 1447 ***K.pneumoniae*** – imipenem and meropenem disk diffusion



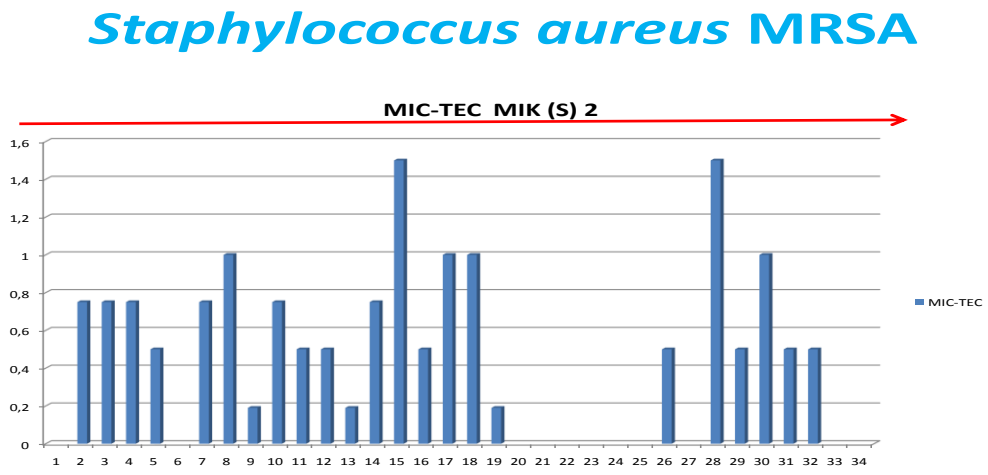
Slika-*Figure 6.*
Soj 03/13 NEQAS 1447 ***K.pneumoniae*** – cefzatidim i ceftriakson, disk difuzija
Strain 03/13 NEQAS 1447 ***K.pneumoniae*** – cefzatidim and ceftriakson, disk diffusion



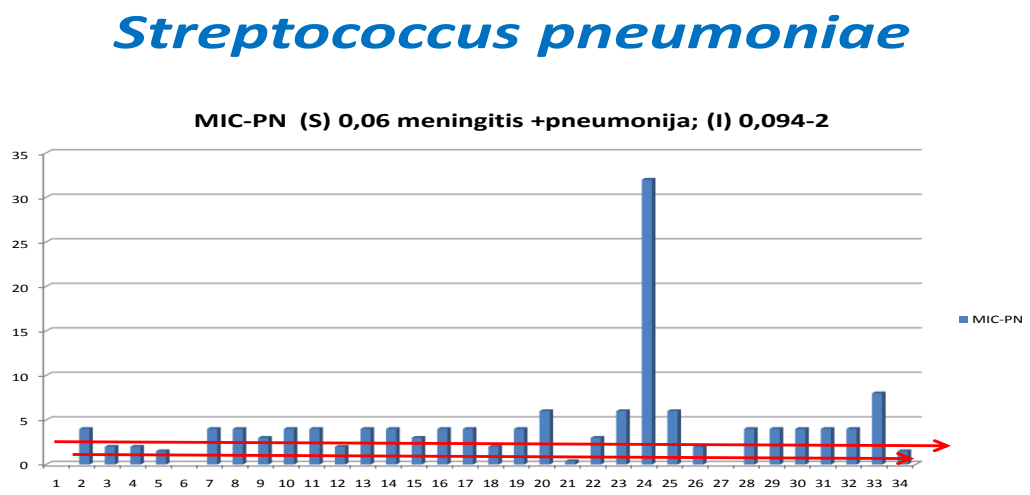
Slika-*Figure 7.*
Soj 04/13 NEQAS 1448 ***S.aureus*** – MIK vankomicin
Strain 04/13 NEQAS 1448 ***S.aureus*** – MIC vankomycin



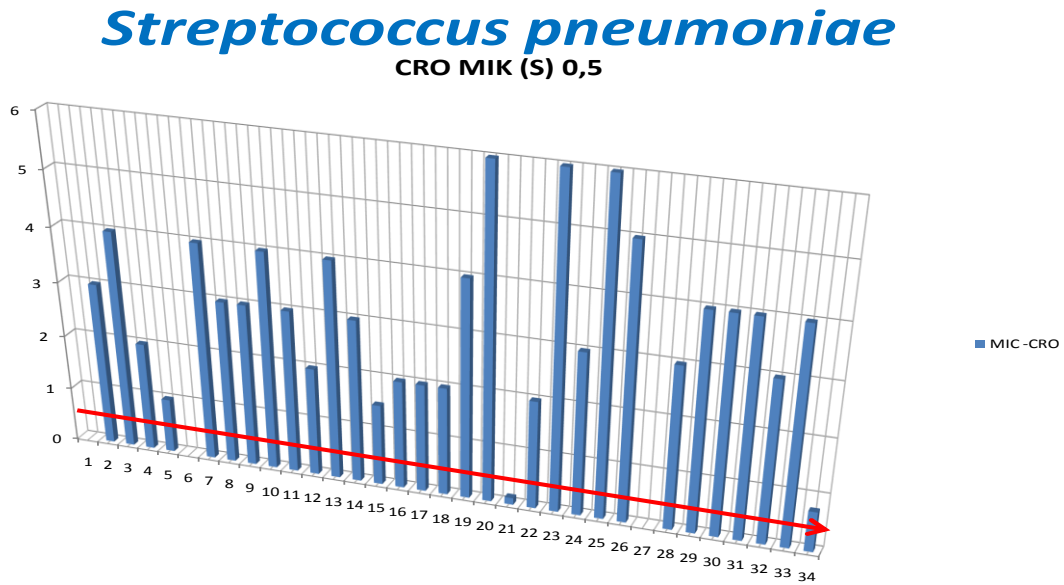
Slika-*Figure 8.*
Soj 04/13 NEQAS 1448 ***S.aureus*** – MIK teikoplanin
Strain 04/13 NEQAS 1448 ***S.aureus*** – MIC teicoplanin



Slika-Figure 9.
Soj 05/13 NEQAS 1449 ***S.pneumoniae*** – MIK penicilin
Strain 05/13 NEQAS 1449 ***S.pneumoniae*** – MIC penicillin



Slika-*Figure 10.*
Soj 05/13 NEQAS 1449 ***S.pneumoniae*** – MIK ceftriakson
Strain 05/13 NEQAS 1449 ***S.pneumoniae*** – MIC ceftriaxone



Tablica-*Table 1.*
Soj 05/13 NEQAS 1449 ***S.pneumoniae*** – klinička interpretacija,penicilin i cefftriakson
Strain 05/13 NEQAS 1449 ***S.pneumoniae*** – clinical interpretation, penicillin and cefftriaksone

Streptococcus pneumoniae

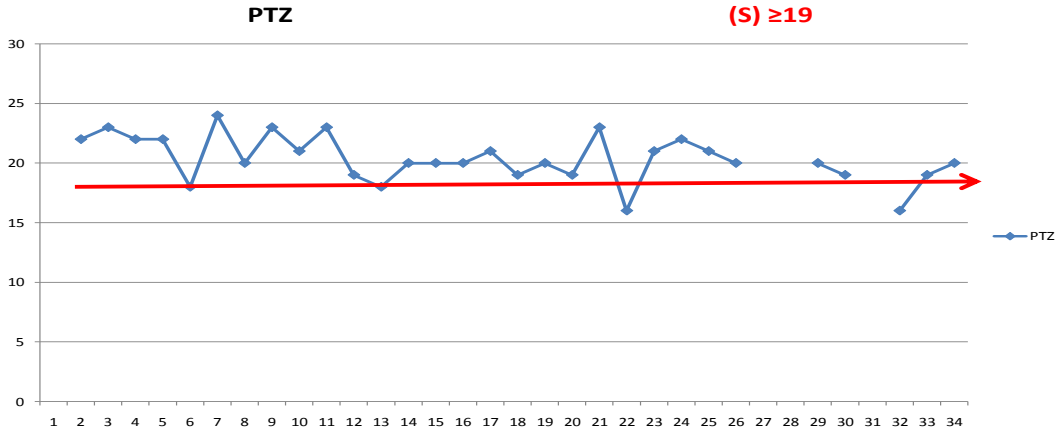
UKNEQAS interpretacija

PEN R;R CRO R;R

S.pneumoniae	Interpretacija			Broj laboratorija
PEN	R	I	S	
- pneumonija	23	3	6	32/33
- meningitis	32	0	0	32/33
CRO	R	I	S	
- pneumonija	22	7	2	31/33
- meningitis	24	6	1	31/33

Slika-*Figure 11.*
Soj 06/13 NEQAS 1450 ***P.aeruginosa*** – piperacilin-tazobaktam, disk difuzija
Strain 06/13 NEQAS 1450 ***P.aeruginosa*** – piperacillin-tazobactam, disk diffusion

Pseudomonas aeruginosa



Zbirni prikaz rezultata za kontrolu/Summary reports EQC: JESEN 2013.

Laboratorij	Ab	Ec	Kp OXA-48	Sa MRSA	Sp,klin.interpretacija/clinic. interpretation	Pa
1 HR001	+	+	+	+	+*CRO	+
2 HR002	+	+	+ MEM *	+CM ind.	+	+
3 HR003	+ID	+	+	+	+ *PEN	+
4 HR004	+ID	+	+	+	+ *PEN,*CRO	+
5 HR005	+	+	+*IMI/MEM	+	+*PEN,*CRO	+
6 HR006	+	+	+*IMI/MEM	+	+*PEN,*CRO	+ *TZP R
7 HR007	+	+	+*IMI/MEM	+CM ind.	+	+
8 HR008	+	+	+	+	+	+
9 HR009	+	+	+*IMI,MEM	+CM ind.	+	+
10 HR010	+	+	+	+	+	+
11 HR011	+	+	+	+	+	+
12 HR012	+	+	+	+	+*PEN,*CRO	+
13 HR013	+	+	+*IMI,MEM	+	+	+
14 HR014	+	+	+	+	+	+
15 HR015	+	+	+*CAZ*MEM	+CM ind.	+ *CRO	+
16 HR016	+	+	+	+	+	+
17 HR017	+	+	+	+	+	+
18 HR018	+	+	+*IMI/MEM	+	+*PEN,*CRO	+
19 HR019	+	+	+*IMI/MEM	+	+	+
20 HR020	+	+	+*IMI/MEM	+	+	+
21 HR021	+	+	+*IMI/MEM	+ CM ind	+*PEN,*CRO	+
22 HR022	+	+	+*MEM	+ CM ind	+*CRO	+
23 HR023	+ID	+	+*IMI/MEM	+	+	+
24 HR024	+ID	+	+	+ CM ind	+	+
25 HR025	+ID	+	+*IMI/MEM	+	+	+
26 HR026	+	+**AMCR	+*IMI/MEM	+ CM ind	+*PEN-pneumo	+
27 HR027						
28 HR028	+	+	+*IMI/MEM	+	+	+
29 HR029	+	+	+	+	+	+
30 HR030	+	+	+	+	+	+
31 HR031	+	+	+*IMI/MEM	+	+	+
32 HR032	+	+	+	+	+	+**TZP
33 HR033	+	+	+*IMI/MEM	+ CM ind	+	+
34 HR034	+	+	+*IMI/MEM	+ CM ind	+*PEN,*CRO	+

GREŠKA/error:*manja/minor **velika/major ***vrlo velika/very major ****greška u interpretaciji/interpretation error

POGLAVLJE/CHAPTER 6.

GENOTIPIZACIJA KLINIČKIH IZOLATA VRSTE *ACINETOBACTER BAUMANNII* I NJIHOVA SPOSOBNOST STVARANJA BIOFILMA MULTICENTRIČNO ISTRAŽIVANJE

GENOTYPING OF CLINICAL ISOLATES OF *ACINETOBACTER BAUMANNII* SPECIES AND THEIR ABILITY TO FORM BIOFILM MULTICENTER STUDY

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GENOTIPIZACIJA KLINIČKIH IZOLATA VRSTE *ACINETOBACTER BAUMANNII* I NJIHOVA SPOSOBNOST STVARANJA BIOFILMA MULTICENTRIČNO ISTRAŽIVANJE

CILJ ovog multicentričnog istraživanja bio je ispitati pripadaju li klinički izolati vrste *Acinetobacter baumannii* prikupljeni iz različitih zdravstvenih ustanova južnog dijela Hrvatske različitim genotipovma, te imaju li različitu sposobnost stvaranja biofilma ovisno o pripadnosti genotipu (klonu), podrijetlu izolata (odjelu ustanove i vrsti kliničkog uzorka iz kojeg su izdvojeni) i u odnosu na osjetljivost na različite antibiotike.

Istraživanjem je obuhvaćeno ukupno 133 klinička izolata bakterije *A. baumannii*, koji su prospektivno prikupljeni zahvaljujući suradnji s Odborom za praćenje rezistencije bakterija na antibiotike pri Akademiji medicinskih znanosti Hrvatske, iz 6 gradova južnog dijela Hrvatske (Pule, Rijeke, Zadra, Šibenika, Splita, Dubrovnika). Za vrijeme godišnjeg tromjesečnog razdoblja praćenja osjetljivosti bakterija na antibiotike od 1. 10. do 31. 12. 2009. godine. svi izolati vrste *A. baumannii* dobiveni tijekom rutinskog rada mikrobioloških laboratorija iz 8 različitih zdravstvenih ustanova (ZZJZ Pula, KBC Rijeka, NZJZ Rijeka, ZZJZ Zadar, ZZJZ Šibenik, KBC Split, NZJZ Split, ZZJZ Dubrovnik), transportirani su (pohranjeni u dubokom agaru) u Klinički zavod za mikrobiologiju i parazitologiju Kliničkoga bolničkog centra Split. Izolati su potom razvrstani po gradovima i odjelima zdravstvenih ustanova u kojima su izolirani, te po vrsti biološkog materijala iz kojeg su izdvojeni.

Kriterij za uključivanje u istraživanje bio je pripadnost prikupljenih kliničkih izolata vrsti *A. baumannii*. Nakon detaljne analize uzoraka, 24 ih je isključeno iz obrade, jer se radilo o „copy“ sojevima istih pacijenata, te je u istraživanje uključeno ukupno 109 kliničkih izolata vrste *A. baumannii*.

Nakon postupaka izolacije i identifikacije klasičnim i komercijalnim testovima za identifikaciju nefermentativnih bakterija API 20NE i VITEK 2 (bioMerieux, Francuska), testirana je osjetljivost izolata na antibiotike disk difuzijskom metodom i određivanjem minimalne inhibitorne koncentracije (MIK). Svi prikupljeni izolati vrste *A. baumannii* genotipizirani su metodom elektroforeze u pulsirajućem polju (engl. *pulsed field gel electrophoresis* PFGE) kako bi se utvrdila zastupljenost pojedinih genotipova (klonova) u izolatima s pojedinih odjela i zdravstvenih ustanova 6 gradova južnog dijela Hrvatske. Svim prikupljenim izolatima ispitana je sposobnost stvaranja biofilma *in vitro* prema publiciranim protokolima, te smo promatrali je li ova sposobnost ovisna o genotipu, o podrijetlu izolata i osjetljivosti na antibiotike.

Rezultati ovog multicentričnog istraživanja prikazani su u priloženoj Tablici 1.

Tablica 1. Popis svih obrađenih izolata bakterije *Acinetobacter baumannii* (n=109)

red. br	grad	odjel	vrsta uzorka	genotipizacija	biofilm OD	BIOFILM	TZP	CAZ	FEP	IMI	MEM	AM	GN	CIP	COL	SAM	MDR
1	Pula	JIL	AT/AB/BAL	EU 1	0,611	slabo stvara	R	R	R	U	U	S	R	R	S	S	ne
2	Pula	JIL	obrisak rane	EU 1	1,118	jako stvara	R	R	R	U	U	S	R	R	S	S	ne
3	Pula	kirurgija	obrisak rane	EU 1	0,038	ne stvara	R	R	R	R	U	S	R	R	S	S	ne
4	Pula	kirurgija	likvor	EU 1	0,452	slabo stvara	R	R	U	U	U	S	R	R	S	S	ne
5	Pula	JIL	AT/AB/BAL	EU 1	0,497	slabo stvara	R	R	R	U	U	S	R	R	S	S	ne
6	Pula	JIL	AT/AB/BAL	EU 1	0,497	slabo stvara	R	R	R	U	U	S	R	R	S	S	ne
7	Pula	JIL	kateter (urin)	EU 1	1,115	jako stvara	R	R	R	R	U	U	R	R	S	S	ne
8	Pula	JIL	likvor	EU 1	1,3	jako stvara	R	R	R	U	U	U	R	R	S	S	ne
9	Pula	JIL	likvor	EU 1	1,3	jako stvara	R	R	R	U	U	U	R	R	S	S	ne
10	Pula	JIL	likvor	EU 1	1,3	jako stvara	R	R	R	U	U	U	R	R	S	S	ne
11	Pula	JIL	AT/AB/BAL	EU 1	0,735	slabo stvara	R	R	R	U	U	U	R	R	S	S	ne
12	Pula	JIL	AT/AB/BAL	EU 1	1,206	jako stvara	R	R	R	U	U	U	R	R	S	S	ne
13	Pula	JIL	AT/AB/BAL	EU 1	1,206	jako stvara	R	R	R	U	U	U	R	R	S	S	ne
14	Pula	JIL	kateter (urin)	EU 1	0,447	slabo stvara	R	R	R	U	U	U	R	R	S	S	ne
15	Pula	JIL	AT/AB/BAL	EU 1	1,206	jako stvara	R	R	R	U	U	U	R	R	S	S	ne
16	Pula	opća medicina	obrisak rane	EU 1	0,084	ne stvara	S	S	S	S	S	S	S	R	S	S	ne
17	Pula	kirurgija	obrisak rane	EU 1	0,959	slabo stvara	R	R	R	U	U	R	R	R	S	S	**da**
18	Pula	opća medicina	obrisak rane	EU 1	0,568	slabo stvara	R	R	U	S	S	S	S	R	S	S	ne
19	Pula	interna	AT/AB/BAL	EU 1	0,519	slabo stvara	R	R	R	U	U	S	R	R	S	S	ne
20	Pula	zarazno	obrisak rane	EU 1	0,752	slabo stvara	R	R	R	U	U	S	R	R	S	S	ne
21	Pula	opća medicina	obrisak rane	EU 1	0,965	slabo stvara	R	R	R	U	U	S	R	R	S	S	ne

red. br	grad	odjel	vrsta uzorka	genotipizacija	biofilm OD	BIOFILM	TZP	CAZ	FEP	IMI	MEM	AM	GN	CIP	COL	SAM	MDR
22	Pula	JIL	AT/AB/BAL	EU 1	1,387	jako stvara	S	S	S	S	S	S	S	S	S	S	ne
23	Pula	JIL	obrisak rane	EU 1	0,578	slabo stvara	S	S	S	S	S	S	S	S	S	S	ne
24	Pula	JIL	likvor	EU 1	1,3	jako stvara	R	R	R	U	U	U	R	R	S	S	ne
25	Split	JIL	krv	EU 2	0,194	ne stvara	R	R	R	R	R	R	R	R	S	R	**da**
26	Rijeka	plućno	AT/AB/BAL	EU 1	0,451	slabo stvara	S	S	S	S	S	S	S	S	S	S	ne
27	Rijeka	plućno	AT/AB/BAL	EU 1	1,579	jako stvara	S	S	S	S	S	S	S	S	S	S	ne
28	Rijeka	neonatologija	AT/AB/BAL	ostali klonovi	0,117	ne stvara	S	S	S	S	S	S	S	S	S	S	ne
29	Rijeka	plućno	AT/AB/BAL	EU 1	0,576	slabo stvara	R	R	R	S	S	S	R	R	S	S	ne
30	Rijeka	hematologija	obrisak nosa	ostali klonovi	0,792	slabo stvara	R	R	R	S	U	U	R	S	S	S	ne
31	Rijeka	JIL	obrisak ždrijela	ostali klonovi	0,237	ne stvara	R	R	R	U	R	R	R	R	S	S	**da**
32	Rijeka	kardiologija	pleuralni punktat	ostali klonovi	0,483	slabo stvara	R	R	S	S	S	S	R	R	S	S	ne
33	Rijeka	JIL	AT/AB/BAL	ostali klonovi	0,943	slabo stvara	S	S	S	S	S	S	S	S	S	S	ne
34	Rijeka	opća medicina	urin	ostali klonovi	0,674	slabo stvara	R	R	R	R	R	U	R	R	S	S	**da**
35	Rijeka	opća medicina	urin	ostali klonovi	0,314	ne stvara	S	R	R	S	U	S	R	S	S	S	ne
36	Rijeka	opća medicina	urin	ostali klonovi	0,587	slabo stvara	R	R	R	S	S	S	R	R	S	S	ne
37	Rijeka	opća medicina	obrisak rane	EU 1	0,321	ne stvara	R	R	R	S	S	R	R	R	S	S	**da**
38	Zadar	kirurgija	obrisak rane	EU 1	0,246	ne stvara	R	R	R	U	U	S	R	R	S	S	ne
39	Zadar	kirurgija	obrisak rane	ostali klonovi	0,304	ne stvara	R	R	R	S	S	S	R	R	S	S	ne
40	Zadar	kirurgija	obrisak rane	EU 1	0,828	slabo stvara	R	R	R	S	S	S	R	R	S	S	ne
41	Zadar	opća medicina	obrisak rane	ostali klonovi	0,195	ne stvara	S	S	S	S	S	S	S	S	S	S	ne
42	Zadar	opća medicina	obrisak rane	ostali klonovi	0,283	ne stvara	S	S	U	S	S	S	S	U	S	S	ne

red. br	grad	odjel	vrsta uzorka	genotipizacija	biofilm OD	BIOFILM	TZP	CAZ	FEP	IMI	MEM	AM	GN	CIP	COL	SAM	MDR
43	Zadar	opća medicina	obrisak rane	ostali klonovi	0,387	ne stvara	S	S	S	S	S	S	S	S	S	S	ne
44	Zadar	JIL	obrisak rane	ostali klonovi	0,483	slabo stvara	R	R	R	S	S	S	R	R	S	S	ne
45	Dubrovnik	urologija	obrisak rane	EU 1	0,183	ne stvara	R	R	S	S	R	S	R	R	S	S	ne
46	Dubrovnik	neurologija	kateter (urin)	EU 1	1,035	jako stvara	S	S	S	S	S	S	S	S	S	S	ne
47	Dubrovnik	opća medicina	obrisak rane	ostali klonovi	0,546	slabo stvara	S	S	S	S	S	S	S	S	S	S	ne
48	Dubrovnik	JIL	AT/AB/BAL	EU 1	1,021	jako stvara	R	R	S	S	U	S	S	S	S	S	ne
49	Dubrovnik	kirurgija	obrisak rane	EU 1	1,055	jako stvara	R	R	S	S	U	S	S	S	S	S	ne
50	Dubrovnik	ortopedija	obrisak rane	ostali klonovi	0,387	ne stvara	R	R	R	R	R	S	R	R	S	S	**da**
51	Dubrovnik	opća medicina	urin	ostali klonovi	0,938	slabo stvara	S	S	S	S	S	S	S	S	S	S	ne
52	Dubrovnik	zarazno	obrisak nosa	EU 1	0,72	slabo stvara	R	R	S	S	U	S	S	R	S	S	ne
53	Dubrovnik	kirurgija	obrisak rane	EU 1	0,487	slabo stvara	S	R	R	S	S	S	R	R	S	S	ne
54	Dubrovnik	interna	obrisak nosa	EU 1	0,649	slabo stvara	R	R	R	S	U	S	R	R	S	S	ne
55	Dubrovnik	opća medicina	urin	ostali klonovi	0,535	slabo stvara	S	S	S	S	S	S	S	S	S	S	ne
56	Pula	JIL	likvor	EU 1	1,3	jako stvara	R	R	R	U	U	U	R	R	S	S	ne
57	Pula	JIL	AT/AB/BAL	EU 1	1,206	jako stvara	R	R	R	U	U	U	R	R	S	S	ne
58	Pula	JIL	likvor	EU 1	1,3	jako stvara	R	R	R	U	U	U	R	R	S	S	ne
59	Pula	JIL	AT/AB/BAL	EU 1	1,206	jako stvara	R	R	R	U	U	U	R	R	S	S	ne
60	Pula	JIL	AT/AB/BAL	EU 1	1,206	jako stvara	R	R	R	U	U	U	R	R	S	S	ne
61	Pula	JIL	likvor	EU 1	1,3	jako stvara	R	R	R	U	U	U	R	R	S	S	ne
62	Pula	JIL	AT/AB/BAL	EU 1	0,497	slabo stvara	R	R	R	U	U	S	R	R	S	S	ne
63	Pula	JIL	likvor	EU 1	1,3	jako stvara	R	R	R	U	U	U	R	R	S	S	ne

red. br	grad	odjel	vrsta uzorka	genotipizacija	biofilm OD	BIOFILM	TZP	CAZ	FEP	IMI	MEM	AM	GN	CIP	COL	SAM	MDR
64	Šibenik	JIL	AT/AB/BAL	ostali klonovi	1,131	jako stvara	R	R	U	S	S	S	R	R	S	S	ne
65	Split	JIL	AT/AB/BAL	EU 2	1,275	jako stvara	R	R	R	R	R	R	R	R	S	S	**da**
66	Split	reumatologija	obrisak rane	ostali klonovi	0,454	slabo stvara	U	U	S	S	S	S	R	R	S	S	ne
67	Split	trauma	obrisak rane	EU 2	1,25	jako stvara	U	U	S	S	S	S	R	R	S	S	ne
68	Split	JIL	AT/AB/BAL	ostali klonovi	0,448	slabo stvara	R	R	R	R	R	R	R	R	S	R	**da**
69	Split	JIL	urin	EU 2	0,254	ne stvara	R	R	R	R	R	R	R	R	S	R	**da**
70	Split	JIL	AT/AB/BAL	EU 2	0,241	ne stvara	R	R	R	R	R	R	R	R	S	R	**da**
71	Split	JIL	AT/AB/BAL	EU 2	1,452	jako stvara	R	R	R	R	R	R	R	R	S	R	**da**
72	Split	JIL	AT/AB/BAL	EU 1	0,512	slabo stvara	R	R	R	R	R	R	R	R	S	R	**da**
73	Split	kirurgija	kateter (dren)	EU 2	0,183	ne stvara	R	R	R	R	R	R	R	R	S	R	**da**
74	Split	JIL	obrisak oka	EU 2	1,417	jako stvara	R	R	R	R	R	R	R	R	S	S	**da**
75	Split	kirurgija	AT/AB/BAL	EU 2	0,483	slabo stvara	R	R	R	R	R	R	R	R	S	R	**da**
76	Split	plućno	AT/AB/BAL	EU 1	0,601	slabo stvara	R	R	R	S	S	R	U	R	S	S	ne
77	Split	JIL	obrisak oka	EU 2	0,87	slabo stvara	R	R	R	R	R	R	R	R	S	S	**da**
78	Split	JIL	AT/AB/BAL	EU 2	0,784	slabo stvara	R	R	R	R	R	R	R	R	S	S	**da**
79	Split	JIL	AT/AB/BAL	EU 2	1,397	jako stvara	R	R	R	R	R	R	R	R	S	S	**da**
80	Split	JIL	AT/AB/BAL	EU 2	0,313	ne stvara	R	R	R	R	R	R	R	R	S	R	**da**
81	Split	JIL	kateter (cvk)	EU 2	1,58	jako stvara	R	R	R	R	R	R	R	R	S	S	**da**
82	Split	JIL	obrisak rane	EU 2	0,602	slabo stvara	U	R	R	S	S	R	R	R	S	S	ne
83	Split	JIL	urin	EU 2	0,318	ne stvara	R	R	R	R	R	R	R	R	S	R	**da**
84	Split	JIL	AT/AB/BAL	EU 2	1,651	jako stvara	R	R	R	R	R	R	R	R	S	U	**da**

red. br	grad	odjel	vrsta uzorka	genotipizacija	biofilm OD	BIOFILM	TZP	CAZ	FEP	IMI	MEM	AM	GN	CIP	COL	SAM	MDR
85	Split	JIL	AT/AB/BAL	EU 2	0,11	ne stvara	R	R	R	R	R	R	R	R	S	S	**da**
86	Split	trauma	obrisak rane	EU 2	0,562	slabo stvara	R	R	U	R	R	R	R	U	S	S	ne
87	Split	kirurgija	AT/AB/BAL	EU 2	0,317	ne stvara	R	R	R	R	R	R	R	R	S	R	**da**
88	Split	JIL	sadržaj abdomena	EU 2	0,237	ne stvara	R	R	R	R	R	R	R	R	S	S	**da**
89	Split	JIL	AT/AB/BAL	EU 2	0,743	slabo stvara	R	R	R	R	R	R	R	R	S	S	**da**
90	Split	opća medicina	obrisak rane	EU 2	0,895	slabo stvara	S	R	U	S	S	S	R	R	S	S	ne
91	Split	JIL	AT/AB/BAL	EU 2	0,909	slabo stvara	R	R	R	R	R	R	R	R	S	R	**da**
92	Split	JIL	AT/AB/BAL	EU 2	1,561	jako stvara	R	R	R	R	R	R	R	R	S	R	**da**
93	Split	JIL	AT/AB/BAL	EU 2	0,296	ne stvara	R	R	R	R	R	R	R	R	S	R	**da**
94	Split	JIL	AT/AB/BAL	EU 2	0,88	slabo stvara	R	R	R	R	R	R	R	R	S	S	**da**
95	Split	JIL	kateter (cvk)	EU 1	0,281	ne stvara	R	R	R	S	S	R	S	R	S	S	ne
96	Split	JIL	AT/AB/BAL	EU 2	1,097	jako stvara	R	R	R	R	R	R	R	R	S	S	**da**
97	Split	JIL	obrisak oka	EU 2	0,762	slabo stvara	R	R	R	R	R	R	R	R	S	S	**da**
98	Split	neurolog	urin	EU 1	0,195	ne stvara	U	R	R	S	S	R	R	R	S	S	ne
99	Šibenik	JIL	AT/AB/BAL	ostali klonovi	1,09	jako stvara	R	R	R	S	U	S	R	R	S	S	ne
100	Šibenik	JIL	krv	ostali klonovi	1,12	jako stvara	R	R	R	S	U	S	R	R	S	S	ne
101	Šibenik	JIL	AT/AB/BAL	ostali klonovi	1,32	jako stvara	R	R	R	S	U	S	R	R	S	S	ne
102	Šibenik	JIL	AT/AB/BAL	ostali klonovi	0,386	ne stvara	R	R	R	R	U	S	R	R	S	S	ne
103	Šibenik	JIL	urin	EU 1	0,729	slabo stvara	R	R	R	U	U	R	R	R	S	S	**da**
104	Šibenik	JIL	obrisak rane	EU 1	0,75	slabo stvara	R	R	R	S	U	S	R	R	S	S	ne
105	Šibenik	JIL	AT/AB/BAL	EU 1	0,355	ne stvara	R	R	R	R	U	U	R	U	S	S	ne

red. br	grad	odjel	vrsta uzorka	genotipizacija	biofilm OD	BIOFILM	TZP	CAZ	FEP	IMI	MEM	AM	GN	CIP	COL	SAM	MDR
106	Šibenik	JIL	AT/AB/BAL	EU 1	0,563	slabo stvara	R	R	R	S	R	S	S	R	S	S	ne
107	Šibenik	hemodijaliza	obrisak uha	EU 1	0,574	slabo stvara	R	R	R	S	R	S	R	R	S	S	ne
108	Šibenik	JIL	obrisak rane	EU 1	0,311	ne stvara	R	R	R	R	U	S	R	R	S	S	ne
109	Šibenik	JIL	AT/AB/BAL	EU 1	0,926	slabo stvara	R	R	R	U	R	S	S	R	S	S	ne

JIL-jedinica intenzivnog liječenja, AT/AB/BAL-aspirat traheje, aspirat bronha,bronhoalveolarni lavat, EU I europski klon I, EU II europski klon II, OD-optical density
TZP-piperacilin/tazobaktam, CAZ-ceftazidim, FEP-cefepim, IMI-imipenem, MEM-meropenem, AM-amikacin, GN-gentamicin, CIP-ciprofloksacin, SAM-ampicilin/sulbaktam, MDR-*multidrug* rezistentan izolat, S= osjetljivi izolati; U= umjereno osjetljivi; R= rezistentni izolati

ZAKLJUČCI: U vrijeme istraživanja jedino je u Splitu utvrđeno postojanje klona EU II vrste *A. baumannii*, za koji je potvrđena veća rezistencija na antibiotike i *multidrug* rezistencija. Veliki broj izolata *A. baumannii* pokazao je sposobnost stvaranja biofilma, a ispitana sposobnost najčešća je kod izolata dobivenih iz jedinice intenzivnog liječenja i iz kliničkog uzorka aspirata traheje, bronha i bronhoalveolarnog lavata, te kod onih koji su bili osjetljivi ili umjereno osjetljivi na antibiotike.

GENOTYPING OF CLINICAL ISOLATES OF *ACINETOBACTER BAUMANNII* SPECIES AND THEIR ABILITY TO FORM BIOFILM MULTICENTER STUDY

The scope of this multicenter study was to investigate if clinical isolates of the *A. baumannii* sampled from various medical institutions in the south Croatia appertain to various genotypes, and if they had various abilities to form biofilm depending on genotypes (clones), origin of tested isolates (departments from which samples are taken and types of samples that give isolates) and resistance to antibiotics.

This study has analyzed in total 133 isolates of the *A. baumannii* sampled prospectively in 6 cities of the south Croatia (Pula, Rijeka, Zadar, Šibenik, Split, Dubrovnik), from 8 medical institutions (Public Health Institutes: Pula, Rijeka, Zadar, Šibenik, Split, Dubrovnik and University Hospital Centers: Rijeka, Split), obtained during the process of monitoring the bacterial resistance to antibiotics, from 01 October until 31 December 2009 (quarterly monitoring period), performed in cooperation with the Croatian Committee for Antibiotic Resistance Surveillance of the CAMS (Croatian Academy of Medical Sciences).

The isolates were stored in nutrient agar and transported to the Clinical Department for Microbiology and Parasitology of the University Hospital Centre Split. Isolates were then sorted by cities and departments of medical institutions in which they are taken, and by the type of biological material from which they were isolated. A criterion for inclusion in the study was the association of collected clinical isolates to *A. baumannii* species.

After detailed analysis of the samples, 24 were excluded from the process, because they were "copy" strains of the same patients, and finally the study included a total of 109 clinical isolates of *A. baumannii*.

After their isolation and identification through standard and commercial tests for the identification of non-fermenting bacteria API 20NE and VITEK 2 (bioMerieux, France), the susceptibility of isolates to antibiotics was tested by applying a disk diffusion method and by determining the minimum inhibitory concentrations (MICs). All the sampled isolates of the *A. baumannii* strain were genotyped by using the PFGE method (*pulsed field gel electrophoresis*) to determine the existence of individual genotypes (clones) in isolates from individual departments and medical institutions in six cities of the south Croatia, according to published protocols. The ability to form biofilm *in vitro* was tested for all the sampled isolates of the *A. baumannii* strain, according to published protocols, and we have observed if this ability depends on genotypes, origin of tested isolates and resistance to antibiotics.

The results of this multicenter study are shown in the table.

CONCLUSION:

During the research, the existence of EU II clone of the *A. baumannii* strain was found only in one hospital (University Hospital Centre Split) and EU II clone was also proved to be more resistant to antibiotics and multidrug resistant. A great number of isolates formed biofilm, which was observed more frequently in isolates recovered from intensive care units and from clinical samples of tracheal, bronchial aspirates and bronchoalveolar lavage as well as in those susceptible or moderately susceptible to antibiotics.