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ANTIBIOTIC RESISTANCE OF GRAM-POSITIVE BACTERIA AT THE SESTRE MILOSRDNICE UNIVERSITY HOSPITAL

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SUMMARY – The study was performed to provide current resistance data on gram-positive pathogens at the Sestre milosrdnice University Hospital in Zagreb. The organisms selected for monitoring of antimicrobial resistance to different antibiotics included *Streptococcus pneumoniae, Streptococcus pyogenes,* methicillin sensitive and resistant *Staphylococcus aureus,* and *Enterococcus* sp., isolated from different clinical specimens of hospitalized adults and children from various Hospital wards. The antibiotic sensitivity testing was performed by disk diffusion and E-test method. Resistance of *Streptococcus pneumoniae* to penicillin was found to be of great importance. It was quite common but moderate, so penicillin remained an appropriate choice in empirical therapy for presumed pneumococcal respiratory infections. Resistance of *Streptococcus pyogenes* to azithromycin was also quite significant, posing a question whether macrolides were an appropriate choice in empirical therapy for presumed *Streptococcus pyogenes* tonsillopharyngitis. As many as 38% of *Enterococcus* sp. could not be treated by a combination of penicillin or glycopeptide and gentamicin because of the high – lerel resistance to gentamicin. Resistance to antibiotics in methicillin sensitive *Staphylococcus aureus* was not observed. Antibiotic resistance data on gram-positive pathogens influence the choice of local empirical therapy and allow for a more rational use of antibiotics at a hospital.

Key words: *Gram positive bacteria, drug* effects; Drug resistance, microbial; Bacterial Infections, microbiology;

Introduction

Antibiotic resistance is among the most challenging problems in microbiology and clinical medicine. Bacteria have a remarkable ability to mutate and acquire resistant genes from other organisms and thereby develop resistance to antibiotics. When an antibiotic is used, the selective pressure exerted by the drug favors the growth of organisms with mutations that allow them to resist the drug action. The extensive use of antibiotics has resulted in the emergence of drug resistance that threatens at the beginning of the new millennium[1]. Examples of clinically important gram-positive bacteria that are rapidly developing resistance to available antibiotics include bacteria that cause pneumonia, ear infections, and meningitis (e.g., *Streptococcus pneumoniae*)[2], skin, bone, lung and bloodstream infections (e.g., *Staphylococcus aureus*)[3], urinary tract and surgical wound infections (enterococci)[4].

The study was performed to provide current resistance data on gram-positive pathogens at the Sestre milosrdnice University Hospital in Zagreb.

Material and Methods

Antibiotic resistance of gram-positive bacteria was monitored for six consecutive months (from January 1 till June 30, 2000). The organisms selected for surveillance

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included *Streptococcus pneumoniae, Streptococcus pyogenes* (beta-hemolytic streptococcus group A), *Enterococcus* sp., methicillin sensitive *Staphylococcus aureus*, and methicillin resistant *Staphylococcus aureus*. These organisms were isolated from different clinical specimens of adult and pediatric inpatients from different Hospital wards. Duplicate or multiple isolates of the selected species were not excluded from the study.

All organisms were identified according to colony morphology and Gram stain. Identification of *Streptococcus pneumoniae* was confirmed by optochin susceptibility test. Bacitracin susceptibility test was used in identification of *Streptococcus pyogenes*. Esculin hydrolysis test was used in identification of enterococcal species. DNase test and slide coagulase test were used for identification of *Staphylococcus aureus*[5].

Antibiotic sensitivity testing was performed by the disk diffusion method according to National Committee for Clinical Laboratory Standard (NCCLS) procedures. Antibiotics were selected individually for each of the organisms selected. Sensitivity of *Streptococcus pneumoniae* to penicillin was determined by 1-μg oxacillin test. E-test penicillin G method was used for the pneumococcal strains that were resistant to oxacillin. In oxacillin resistant strains, the sensitivity of *Streptococcus pneumoniae* to ceftriaxone was determined by E-test ceftriaxone method. The sensitivity of *Staphylococcus aureus* to beta-lactams was determined by 1-μg oxacillin disk. In enterococcal species, the high-level resistance to aminoglycosides was determined by 120-μg gentamicin disk[6,7].

Results

Percentage of resistance of different bacteria to different antibiotics is presented in Tables 1-5.

Streptococcus pneumoniae (Table 1a, 1b). Most pneumococcal isolates were isolated from respiratory specimens and nasopharyngeal swabs, in pediatric patients, pediatric rehabilitation patients, ear-nose-throat (ENT) patients, and pulmonology patients. The disk diffusion method revealed pneumococcal resistance to penicillin to be quite common, with an average of 53%. Approximately 97% of penicillin resistant pneumococcal strains showed moderate resistance (MIC 0.12-1.0 μg/ml) and only 3% high resistance (MIC >2 μg/ml) to penicillin when tested by E-test penicillin G method. The average resistance to cotrimoxazole was 47%, to azithromycin 22%, and to tetracycline 33%. The average resistance to ceftriaxone as determined in oxacillin resistant strains was 22%. Likewise penicillin resistance, ceftriaxone resistance in pneumococcal strains was moderate (MIC 1 mg/ml) when tested by E-test ceftriaxone method.

Table 1a. Percentage of resistance in Streptococcus pneumoniae

Antibiotic	PEN	AZI	SXT	TE
No. of isolates tested	217	218	225	218
Ward:				
Pediatrics	49	23	48	39
Children rehabilitation	42	15	54	8
Ear-nose-throat	63	32	55	41
Pulmonology	50	8	33	17
Total	53	22	47	33

PEN=penicillin; AZI=azithromycin; SXT=cotrimoxazole; TE=tetracycline

Table 1b. Percentage of ceftriaxone resistance in oxacillin resistant Streptococcus pneumoniae

Antibiotic	CRO
No. of isolates tested	65
Ward:	
Pediatrics	6
Children rehabilitation	8
Ear-nose-throat	9
Pulmonology	0
Others	25
Total	22

CRO=ceftriaxone

Streptococcus pyogenes (Table 2). Most isolates of *Streptococcus pyogenes* were isolated from throat and nasopharyngeal swabs in pediatric patients, ENT patients, pediatric rehabilitation patients, and internal medicine patients. The average azithromycin resistance was 17%. Penicillin resistance did not occur.

Table 2. Percentage of penicillin and azithromycin resistance in Streptococcus pyogenes

Antibiotic	PEN	AZI
No. of isolates tested	22	18
Ward:		
Pediatrics	0	20
Children rehabilitation	0	25
Ear-nose-throat	0	0
Internal medicine	0	0
Others		
Total	0	17

PEN=penicillin; AZI=azithromycin

Table 3. Percentage of resistance in Enterococcus sp.

Antibiotic	AMP	GE	VA
No. of isolates tested	421	419	116
Ward:			
Internal medicine	7	44	0
Surgery	8	37	0
Neurology	7	28	0
Pediatrics	19	33	0
Urology	10	24	0
Dermatology	6	35	0
Others			
Total	9	38	0

AMP=ampicillin; GE=gentamicin; VA=vancomycin

Enterococcus sp. (Table 3). Most isolates of *Enterococcus* sp. were isolated from urine, wound swabs, and blood specimens from internal medicine, surgery, neurology, pediatrics, urology and dermatology patients. Nine percent of all enterococcal isolates were resistant to ampicillin, while an average of 38% isolates showed high level gentamicin resistance. Resistance to vancomycin did not occur.

Methicillin sensitive *Staphylococcus aureus* (Table 4). Most isolates of methicillin sensitive *Staphylococcus aureus* were isolated from wound swab, tracheal aspirate, nasopharyngeal swabs and blood specimens in pediatrics, internal medicine, surgery, dermatology, ENT, and neurology patients. An average resistance to clindamycin (4%), azithromycin (7%), cotrimoxazole (3%), ciprofloxacin (16%), gentamicin (6%) and mupirocin (13%) was recorded. Resistance to rifampicin and vancomycin did not occur.

Methicillin resistant *Staphylococcus aureus* (Table 5). Most isolates of methicillin resistant *Staphylococcus aureus* were isolated from wound swabs, blood, tracheal aspirate and other respiratory specimens in surgery, internal medicine, neurology, pulmonology, and neurosurgery patients. Methicillin resistant strains often showed multiple resistance, with an average resistance of 98% to clindamycin, 99% to both azithromycin and ciprofloxacin, and 100% to gentamicin. Average resistance to rifampicin was 18%, and to cotrimoxazole 2%. Resistance to mupirocin and vancomycin was not recorded.

Table 4. Percentage of resistance in methicillin sensitive Staphylococcus aureus

Antibiotic	OX	CC	RIF	AZI	SXT	CIP	GE	MUP	VA
No. of isolates tested	309	244	23	299	308	50	284	107	27
Ward:									
Pediatrics	0	0	0	9	0	2	0	1	0
Internal medicine	0	4	0	3	4	4	5	3	0
Surgery	0	3	0	5	3	3	3	0	0
Dermatology	0	0	0	10	8	0	15	23	0
Ear-nose-throat	0	22	0	28	11	6	11	6	0
Neurology	0	0	0	0	0	0	9	9	0
Others									
Total	0	4	0	7	3	16	6	13	0

OX=oxacillin; CC=clindamycin; RIF=rifampicin; AZI=azithromycin; SXT=cotrimoxazole; CIP=ciprofloxacin; GE=gentamicin; MUP=mupirocin; VA=vancomycin

Table 5. Percentage of resistance in methicillin resistant Staphylococcus aureus

Antibiotic	OX	CC	RIF	AZI	SXT	CIP	GE	MUP	VA
No. of isolates tested	247	172	131	218	250	155	242	60	211
Ward:									
Surgery	100	67	8	85	1	59	100	0	0
Internal medicine	100	82	15	85	4	70	100	0	0
Neurology	100	42	0	95	0	63	100	0	0
Pulmonology	100	60	27	87	7	67	100	0	0
Neurosurgery	100	100	0	100	0	25	100	0	0
Total	100	98	18	99	2	99	100	0	0

OX=oxacillin; CC=clindamycin; RIF=rifampicin; AZI=azithromycin; SXT=cotrimoxazole; CIP=ciprofloxacin; GE=gentamicin; MUP=mupirocin; VA=vancomycin

Discussion

This type of antibiotic resistance surveillance study provides data of great concern for clinicians. These data warn of the emergence of resistance among gram-positive pathogens. Our results showed percentage resistance of different gram-positive pathogens to different antibiotics. There were differences in the antibiotic resistance of different gram-positive pathogens from different Hospital wards. Data obtained by local antibiotic resistance surveillance are used to design empirical therapy at the hospital as a whole or at a particular ward, at the same time introducing a more rational use of antibiotics. This was the final goal of our study, i.e. to design local empirical therapy, thus to discourage misuse and overuse of antibiotics in empirical therapy.

Of great importance is the problem of *Streptococcus pneumoniae* resistance to penicillin. Moderate resistance of *Streptococcus pneumoniae* to penicillin at the Hospital was found to be very common, with an average of 53%. High resistance to penicillin was quite infrequent (3%). Because of as yet uncommon high penicillin resistance of pneumococcal isolates at the Hospital, penicillin remained an appropriate choice in empirical therapy for presumed pneumococcal respiratory infections. An average of 22% of *Streptococcus pneumoniae* strains resistant to azithromycin point to the problem of an increasing resistance to macrolides, and raise the question of whether macrolides should be used as antibiotics in empirical therapy for presumed pneumococcal infections. National data on *Streptococcus pneumoniae* resistance were issued by the Croatian Committee for Antibiotic Resistance Surveillance for the year 1997. At the national level, the average resistance to penicillin was 37% and to azithromycin 12%, however, quite a high resistance was recorded in some parts of Croatia[8]. At our Hospital, moderate resistance to ceftriaxone among oxacillin resistant strains of *Streptococcus pneumoniae* averaged to 22%. In the Latin American Surveillance and Epidemiology Research Study of susceptibility of 1100 pneumococcal strains, Jacobs et al. report on 12% of pneumococcal strains moderately resistant to penicillin to be resistant to ceftriaxone, and even 88% of strains highly resistant to penicillin to be resistant to ceftriaxone[9].

At our Hospital, penicillin resistance was not observed in *Streptococcus pyogenes*, however, an average 17% resistance to azithromycin is considered significant. Therefore, the question should be posed again: are macrolides appropriate antibiotics in empirical therapy for presumed *Streptococcus pyogenes* tonsillopharyngitis?

The average resistance of *Enterococcus* sp. to ampicillin was very similar to that reported at the national level[8], however, the average of 38% of high resistance to gentamicin appears to be quite a disturbing finding, implying that 38% of *Enterococcus* sp. cannot be treated by a combination of penicillin or glycopeptide and gentamicin in case of systemic enterococcal infections. When an enterococcal strain is highly resistant to the aminoglycoside gentamicin, there is no synergism, and combination therapy will not exert bactericidal effect[5]. No isolate resistant to vancomycin was recorded.

Resistance to antibiotics in methicillin sensitive *Staphylococcus aureus* was relatively uncommon. Resistance to mupirocin, an antibiotic for topical treatment of staphylococcal superficial soft tissue infections, showed an av-

erage of 13%. These data differ from the Croatian national data[8], and may have resulted from the overuse of mupirocin at some of the Hospital wards.

Methicillin resistant *Staphylococcus aureus* showed an average resistance of 98% to clindamycin, and 99% to azithromycin and ciprofloxacin each. There was no isolate resistant to vancomycin.

Conclusion

The antibiotic resistance data on gram-positive pathogens at different wards of a hospital will warn clinicians of the presence and emergence of antimicrobial resistance, thus influencing the choice of local empirical therapy and resulting in a more appropriate and more rational use of antibiotics. Clinicians should be kept informed about the problems of drug resistance at their wards and in communities in general, to help them prescribe appropriate drugs and avoid therapeutic failure.

Resistance of gram-positive pathogens will be continuously monitored, and predominant trends and variation in the rate of antimicrobial resistance will hopefully be recognized and interpreted in additional studies.

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References

1. Public Health Service. A public health action plan to combat antimicrobial resistance. Atlanta, GA: Centers for Disease Control and Prevention, 2000.
2. HOFMANN J, CETRON MS, FARLEY MM. The prevalence of drug-resistant *Streptococcus pneumoniae* in Atlanta. N Engl J Med 1995;333:481-6.
3. PANLILIO AL, CULVER DH, GAYNES RP. Methicillin-resistant *Staphylococcus aureus* in U.S. hospitals, 1975 – 1991. Infect Control Hosp Epidemiol 1992;13:582-6.
4. JAJIĆ-BENČIĆ I, BENČIĆ I. Vancomycin-resistant enterococci. Acta Clin Croat 1999;38:33-7.
5. MURRAY PR. Manual of clinical microbiology. 7th Edition. Washington, D.C.: American Society for Microbiology, 1999.
6. NCCLS. Performance standards for antimicrobial susceptibility testing. Eighth Informational Supplement. NCCLS document M100-S8. Pennsylvania: NCCLS, 1998.
7. NCCLS. Performance standards for antimicrobial disk susceptibility tests. Sixth Edition. Approved standards. NCCLS document M2-A6. Pennsylvania: NCCLS, 1997.
8. TAMBIĆ T, TAMBIĆ A, KALENIĆ S, GILIĆ V, KRAKAR B, PAYERL-PAL M, PETANOVIŽ M, PUNDA-POLIŽ V, RADOLOVIŽ Lj, RITTERMAN I, SOKAL A, ŠTERK-KUZMANOVIĆ N, TKALEC N, VUKOVIĆ D. Praženje rezistencije bakterija na antibiotike u Republici Hrvatskoj. Lijec Vjesn 2000;122:160-4.
9. JACOBS MR, APPELBAUM PC, Laser Study Group. Susceptibility of 1100 *Streptococcus pneumoniae* strains isolated in 1997 from seven Latin American and Caribbean countries. Int J Antimicrob Agents 2000;16:17-24.

Sažetak

OTPORNOST GRAM-POZITIVNIH BAKTERIJA NA ANTIBIOTIKE U KLINIČKOJ BOLNICI "SESTRE MILOSRDNICE"

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Cilj istraživanja bio je pratiti otpornost gram-pozitivnih uzročnika na antibiotike u Kliničkoj bolnici "Sestre milosrdnice" u Zagrebu. Otpornost na antibiotike pratila se za sljedeće gram-pozitivne uzročnike: *Streptococcus pneumoniae, Streptococcus pyogenes,* na meticilin osjetljiv i rezistentan *Staphylococcus aureus* te *Enterococcus* sp., koji su izolirani iz kliničkih materijala hospitaliziranih odraslih osoba i djece. Testiranje osjetljivosti na antibiotike provedeno je pomoću difuzijske metode i E-testom. Vrlo je značajan bio problem otpornosti *Streptococcus pneumoniae* na penicilin. Ova je otpornost bila vrlo česta, ali je umjerena, što znači da penicilin i dalje ostaje prikladnim antibiotikom u empirijskoj terapiji pretpostavljenih pneumokoknih respiracijskih infekcija. Otpornost *Streptococcus pyogenes* na azitromicin bila je također značajna, pa se postavlja pitanje jesu li makrolidi prikladni antibiotici u empirijskoj terapiji gnojnih angina. Čak 38% *Enterococcus* sp. ne može se liječiti kombinacijom penicilina ili glikopeptida i gentamicina zbog visoke otpornosti enterokoka na gentamicin. Otpornost na antibiotike kod *Staphylococcus aureus* osjetljivih na meticilin nije bila česta. Otpornost na vankomicin kod *Staphylococcus aureus* otpornih na meticilin nije zabilježena. Podaci o otpornosti gram-pozitivnih uzročnika na antibiotike utječu na izbor lokalne empirijske terapije i omogućavaju racionalniju potrošnju antibiotika u bolnici.

Ključne riječi: *Gram-pozitivne bakterije, učinak lijekova; Otpornost na lijekove, mikrobni; Bakterijske infekcije, mikrobiologija*