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Surveillance of gram-positive cocci infections and drug resistance

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Abstract

In this study, the prevalence of gram-positive cocci isolates and the characteristics of multiple drug resistances in patients were investigated. Antibiotic resistances were determined in the clinical microbiology laboratory with the methodology of the CLSI (2012). The software WHONET5.4 and SPSS13.0 were used for statistical analysis. There were a total of 6211 gram-positive cocci isolates, comprised of 2255 (36.3%) coagulase (-) staphylococci, 1277 (20.6%) staphylococci aureus, 1109 (17.9%) enterococcus faecalis, and 1045 (16.8%) enterococcus faecium. The proportion of Methicillin resistant staphylococcus aureus (MRSA) was 16.6% (212/1277). Methicillin resistant coagulase (-) staphylococci (MRCNS) was 14.1% (318/2255). There were no strains in isolated enterococci resistant to vancomycin, teicoplanin and linezolid. Among the majority of all monitored antibiotics, methicillin resistant staphylococci has much higher drug resistance rate than methicillin sensitive staphylococci ($p<0.05$). Enterococcus faecalis has higher multiple drug resistant rate than enterococcus faecium ($p<0.01$). This research may support the clinicians in prescribing antibiotics properly.

Key words: Staphylococcus, enterococcus, antimicrobial resistance, MRSA, MRCNS.

Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) are isolates of Staphylococcus aureus which have acquired genes encoding antibiotic resistance to all penicillins including methicillin. This resistance is mediated by an altered penicillin binding protein (PBP2a) which is encoded by the Mec A gene (1).They were first discovered in the United Kingdom in 1961(2) but have now become a major clinical problem worldwide (3). It has been reported that isolations of MRSA as well as vancomycin resistant entrococci (VRE) and penicillin resistant streptococcus pneumoniae have been rising continuously in different countries (4). Therefore we studied the isolated 6211 gram positive cocci during 2012 and 2013 in our hospital to understand their drug sensitivities. The result of our studies will help clinicians in choosing appropriate antibiotics.

Materials and methods

Ethics

The sample and detection protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of Chinese Academy of Medical Sciences and Peking Union Medical College. All studies were performed in accordance with national ethic regulations.

Origin of isolated strains

From January 2012 to December 2013, a total of 6211 gram positive cocci were individually isolated at the Beijing Yanhua Hospital.

Susceptibility testing

The antimicrobial susceptibility profile was performed using the disk diffusion method. The antimicrobials agents tested were ampicillin (10μg), amoxycillin/clavulanic (30μg), azithromycin (15μg), chloramphenicol (30μg), ciprofloxacin (5μg), clindamycin (2μg), erythromycin (15μg), gentamicin (120μg), levofloxacin (5μg), linezolid (30μg), minocycline (30μg), nitrofurantoin (300μg), penicillin G (10μg), rifampicin (5μg), sulphamethoxazole/trimethoprim (25μg), teicoplanin (30μg), tetracycline (30μg) and vancomycin (30μg). For isolates resistant according to the screening test, the minimal inhibitory concentration (MIC) for vancomycin was determined using the broth dilution method. All susceptibility tests were performed and interpreted according to guidelines established by the Clinical and Laboratory Standards Institute (CLSI) 2012 (5).

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Table 1. Composition of 2012-2013 year G+ cocci isolates in our local hospital.

Isolates	2012	2013
coagulase (-) staphylococci	1006 (35.3%)	1249 (37.1%)
staphylococci aureus	557 (19.6%)	720 (21.4%)
enterococcus faecalis	520 (18.3%)	589 (17.5%)
enterococcus faecium	466(16.4%)	579 (17.2%)
streptococci	293 (10.3 %)	232 (6.8%)
Total	2842	3369

Statistical methods

All raw data were analysed using the WHONET5.4 software from the WHO antimicrobial resistance monitoring website (http://www.who.int). The $\chi 2$ test of SPSS version 13.0 was applied for calculation. $P<0.05$ is considered to be statistical significant.

Results

Composition of G+ cocci isolates during 2012-2013 year

There was large number of coagulase (-) staphylococci isolated in 2012, and the number increased in 2013. However, the number of streptococci isolates decreased in trend. Staphylococcus aureus grew quickly during these 2 years whereas the number of enterococcus faecium rose gradually. The enterococcus faecalis did not have remarkable changes. The composition of G+ cocci isolates was shown in Table 1.

Antimicrobial resistance of Staphylococci

Isolates from the various time periods did not demonstrate resistance or increases in the MIC of vancomycin and teicoplanin. Methicillin resistant staphylococcus aureus (MRSA) constitutes 16.6% (212/1277); Methicillin resistant coagulase(-) staphylococcus (MRCNS) constitutes 14.1% (318/2255). Methicillin resistant staphylococcus strains (MRS) has much higher resistance rates than Methicillin sensitive staphylococcus strains (MSS) ($P<0.05$). The results were shown in Table 2.

Antimicrobial resistance of enterococci

The resistance rate of enterococcus faecium to chloramphenicol and tetracycline was much higher than enterococcus faecalis. However, the resistance of enterococcus faecium to other antibiotics was less than enterococcus faecalis. There was no observed resistance to teicoplanin or vancomycin from either enterococci. The results were shown in Table 3.

Table 2. Numbers of MRSA/MSSA and MRCNS/MSCNS isolates as well as their resistance to 13 different antibiotics.

Antibiotics	MRSA			MSSA			P
	Number	%R	%I	Number	%R	%I	
amoxycillin	132	92.9	0	118	25.3	0	<0.001
azithromycin	13	100	0	17	74.8	14.1	<0.001
ciprofloxacin	183	97.1	0	172	12.1	9.2	<0.001
clindamycin	175	89.1	2.3	158	47.3	18.7	<0.001
erythromycin	198	91.3	5.6	215	69.6	17.4	<0.001
gentamicin	201	91.7	1.8	214	29.7	2.3	<0.001
levofloxacin	120	90.6	0	134	11.6	1.9	<0.001
linezolid	203	0	0	213	0	0	-
penicillin G	207	100	0	221	94.8	0	<0.001
sulphamethoxazole/trimethoprim	24	26.5	11.2	34	24.3	2.3	0.524
teicoplanin	124	0	0	130	0	0	-
tetracycline	175	75.8	4.8	183	20.6	3.4	<0.001
vancomycin	162	0	0	179	0	0	-
Antibiotic agents	**MRSCoN**			**MSSCoN**			**P**
	Number	**%R**	**%I**	**Number**	**%R**	**%I**	
amoxycillin	64	79.4	0	48	25.3	0	<0.001
azithromycin	21	98	2	11	88	2.7	<0.001
ciprofloxacin	247	86.6	4.5	48	60.4	7.7	<0.001
clindamycin	287	72.2	10.8	115	53	14	<0.001
erythromycin	304	95	2.5	125	87	2.5	<0.001
gentamicin	289	76.9	2.2	118	43.7	2.3	<0.001
levofloxacin	294	75	7.6	114	44.8	17	<0.001
linezolid	301	0	0	109	0	0	-
penicillin G	294	100	0	108	91.8	0	<0.001
sulphamethoxazole/trimethoprim	27	82.4	3.8	12	75.3	2.1	0.035
teicoplanin	131	0	0	54	0	0	-
tetracycline	218	36.3	0.5	43	38.8	3.1	0.0547
vancomycin	235	0	0	49	0	0	-

R: resistance rate; I: Intermediary rate

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Table 3. Comparison of the resistance of enterococcus faecium and enterococcus faecalis to 13 different antibiotics.

Antibiotic	enterococcus faecalis			enterococcus faecium			P value
	Number	%R	%I	Number	%R	%I	
ampicillin	643	26.3	0	582	92.2	0	<0.001
chloramphenicol	144	25.9	6.1	113	14.8	10.9	0.018
erythromycin	853	89.3	8.3	814	96.4	2.1	0.018
gentamicin120	693	42.4	1.1	618	89.6	0.5	<0.001
levofloxacin	653	45.6	10.7	593	93.7	3.1	<0.001
linezolid	974	0	0	938	0	0	--
minocycline	32	31.7	32	23	21.4	18.4	0.001
nitrofurantoin	604	18.4	6.1	584	54.9	5.9	<0.001
penicillin G	743	25.3	0	746	94.3	0	<0.001
rifampicin	203	47	14.1	187	80.2	9.3	<0.001
teicoplanin	435	0	0	341	0	0.2	--
tetracycline	813	58.9	7.2	785	45.3	1.7	0.002
vancomycin	934	0	0	894	0	0.3	--

R: resistance rate; I: Intermediary rate

Discussion

In the recent 20 years, the gram positive cocci have become the major pathogen of hospital infections. It is also one of the major pathogens in the community. Among all kinds of gram positive cocci, staphylococcus is a most dangerous pathogen that could cause several diseases, ranging from minor infections of the skin to wound infections, bacteraemia, and necrotizing pneumonia. International research demonstrated that staphylococcus has always been on the top pathogen causing gram positive cocci hospital infection (6). The data from our studies demonstrated that during 2012-2013 coagulase(-) staphylococcus accounted for most part of isolates in our hospital followed by the staphylococcus aureus and enterococcus whereas streptococcus isolates are lower than 10%. The epidemiological characteristics of S. aureus, especially methicillin-resistant S. aureus, are changing rapidly. Methicillin, the first penicillinase-resistant penicillin, revolutionized the treatment of penicillin-resistant Staphylococcus aureus when introduced into clinical practice in 1959 (7). Within just two years, however, methicillin-resistant strains began to emerge. During the ensuing 5 decades, methicillin-resistant staphylococcus has appeared in hospitals worldwide (8). Methicillin resistant staphylococcus strains constitutes 40.5% of staphylococcus aureus, and 84.8% of coagulase(-) staphylococcus. Locally our hospital in the last 2 years the MRSA isolated was 16.6% whereas the MRCNS was 14.1%, both extremely lower than the national average. Furthermore, in the present study, we analysed the staphylococcus resistance to 13 different antibiotics. Table 2 demonstrated that there was no Vancomycin, Teicoplanin and Linezolid reisistant strains of staphylococcus. After comparison of the resistance rates of MRSA and MSSA, MRCNS and MSCNS are amongst the majority of monitored antibiotics. Methicillin resistant staphylococcus strains has much higher antibiotics resistance than Methicillin sensitive staphylococcus strains ($P<0.05$). Based on this, when treating cocci infections, clinicians should distinguish clearly whether they are methicillin resistant. Regarding the staphylococcus aureus strains, MSSA and MRSA have no statistically significant different resistance rate to sulfanomides ($P>0.05$). This suggests that the clinician can use sulfanomide as empirical therapy for unknown S aureus infections. For coagulase(-) staphylococcus, MSCNS and MRCNS have no statistically significant different resistance rate to tetracyclines, P value is 0.0547($P>0.05$), making the tetracyclines to be the empirical therapy of the choice. As seen in Table 2, MRSA has less resistance rate to sulfanomides, Vancomycin, Teicoplanin and linezolid, being 26.5%, 0%, 0% and 0%, respectively. However, MRSA that has shown multiple drug resistances to the other commonly used antibiotics has resistance rate usually greater than 75%. MSSA has very good sensitivity to the commonly used antibiotics, except for high resistance to the penicillins, macrolides and Clindamycin., but with less than 30% resistance rate to other antibiotics. Therefore these antibiotics may be used as the empirical therapy for the MSSA infections.

Enterococci are widespread in nature and result in different disorders, such as urinary tract infections, intra-abdominal abscesses, wound infections, endocarditis and bacteraemia (9). Although there are at least 30 species of the genus Enterococcus, both E. faecalis and E. faecium are the most common species causing human infections (10). According to the Sentry Antimicrobial Surveillance Program, Enterococci are normal constituents of the human gastrointestinal tract, but nowadays they have been recognized as important pathogens, especially among hospitalized patients. It is worth noting that our hospital the enterococcus faecium isolates had the significant growth during recent 2 years. The possible explanation is that the overuse of third generation of cephalosporins in this area. The enterococci is naturally resistant to the majority of third generation of cephalosporins. As cephalosporins suppress other pathogenic bacterium, the enterococci can grow significantly (11). This then leads to the local enterococci isolates increase. In the recent two years due to more strict management of antibiotic use, clinician rationalises the use of antibiotics based on pathological tests reports. The use of third generation of cephalosporins has declined significantly which allowed the enterococci isolates to decrease. There were similar reports from other countries. In a study conducted in a hospital in Greece, the authors reported a similar increased incidence of E. faecium infections (0.3 in 2002 to 2.4 in 2007), approximately an eight-fold increase (12). The enterococci has complex mechanism for its drug resistance proper-

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ties, owing to their thick cell wall. They can develop naturally resistance, acquired resistance and tolerance (13). The data above has demonstrated that, to the 13 different antibiotics, enterococcus faecium has significantly higher resistance rate than enterococcus faecalis ($P<0.001$). Therefore, they should be differentiated in clinical setting for selecting the appropriate antibiotics. Vancomycin-resistant enterococci (VRE) isolates were first recognized in the 1980s in Europe and USA (14). The prevalence of VRE has been rising across the world every year. Our country also has such reports (15). VRE may cause systemic infection, such as septicaemia, the endocarditis and so on. These can become very difficult to treat. This study has not found any enterococci resistant to Vancomycin, Teicoplanin and Linezolid.

In conclusion, the findings had shown that staphylococcus is the major pathogen for gram positive cocci hospital infections locally in the last 2 years. The tetracyclines and the sulfanomides had good activity against staphylococcus infection locally. No strains of gram positive cocci resistant to Vancomycin, Teicoplanin or Linezolid were isolated. Continuous monitoring on sensitivity and rationalising the use of antibiotics will remain to be the important and effective strategy to ensure the effectiveness of these antibiotics.

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