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A Surveillance of Antimicrobial Resistance in a University-affiliated Hospital in North China in 2012

Fengyan Pei*, Jingxue Chu*,Yan Liu, Lili Wang, Fang Zhang, Mingyu Ji, Cuiming Cao, Yan Wang, Yan Zheng and Yunshan Wang****

Medical Laboratory Diagnostic Center, Jinan Central Hospital
Affiliated to Shandong University, Jinan - 250 013, China.

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Antimicrobial resistance, the ability of bacteria to inhibit the function of antibiotic drugs, has become a public health concern. To investigate susceptibilities of common clinical strains to antimicrobials, 1641 clinical isolates were tested according to Clinical and Laboratory Standards Institute (CLSI) guidelines by an annual Jinan Central Hospital in north China surveillance study (JNCHSS) in 2012. Of which, gram positive strains and gram negative strains accounted for 29.3% and 70.7%, respectively. The prevalence of methicillin-resistant strains was 47.0% in *S. aureus* (MRSA) and 69.8% in *coagulase-negative Staphylococcus* (MRCNS). No *Staphylococcus* strain was found to resist to vancomycin or linezolid. In *Enterobacteriaceae*, extended spectrum β-lactamases (ESBLs) were produced in 60.5% of the *E. coli* strains and 44.7% of the *K. pneumoniae*. More than 30% *P. aeruginosa* strains were only resistant to ticarcililin-clavulanate, aztreonam, imipenem and more than *45%* of *A.baumannii* strains were resistant to all antimicrobials except minocycline. *S.maltophilia* strains were relatively susceptible to sulfamethoxazole-trimethoprim (SXT), minocycline, levofloxacin with lower resistance rate. There were 54.3% of *H. influenzae* which produced β-lactamase. In conclusion, as these data clearly illustrate, we are facing a overwhelming situation of bacterial resistance. Much attention should be paid to periodic surveillance of antibiotic resistance, which is necessary and valuable for antimicrobial therapy. Our research provides a summary of antimicrobial resistance in Jinan Central Hospital.

Key words: Surveillance; Bacterial resistance; Antibiotic resistance.

Antibiotic resistance (AR) threatens human health worldwide [1]. It is well recognized that applications of antibiotics to human clinical therapy, agriculture, aquaculture, and animal husbandry contribute to the emergence and amplification of pathogens due to selective pressure [2, 3]. The immense clinical impact of antimicrobial resistance is usually the result of inappropriate initial antimicrobial therapy [4-5], which may extend a patient's stay in the hospital and expose them to increasing opportunities of antibiotic resistance [6]. There is a direct correlation between antibiotic resistance and patient outcomes, including mortality, period of hospital stay and healthcare costs [7-8]. The situation gets worse as the road extends [9] and nosocomial infections increase. We may soon face the end of the "antibiotic era". The initial and seemingly everlasting success of antibiotics has been challenged by an escalation of resistance mechanisms in bacteria [10].

It was pointed out that microbial surveillance data can guide caregivers who empirically select initial antimicrobial agents for the patients and also support policy makers who address other needs, such as antibiotic

* Fengyan Pei and Jingxue Chu contributed equally to this work.
**Correspondence to: Yunshan Wang and Yan Zheng
Phone: +86-531-85695350;
E-mail: sdjnwys@163.com, zhengdr@hotmail.com

stewardship and cost [11]. Such groups may thus determine which agents are available for caregivers to select and which agents will be recommended for each type of infection in guidelines circulated to caregivers in the area [12].To address this need, JNCHSS evaluated against a recent collection of pathogens isolated from an affiliated hospital of Shandong University in one year. We hope that there were any significant resistance patterns and trends that might help us.

MATERIALS AND METHODS

Collection of bacterial isolations

This study stretched from January to December in 2012. Bacterial isolates were collected from outpatients and inpatients with urinary, respiratory, wound, bloodstream and others infections in our hospital, a tertiary-care hospital with 1700-bed in China. Only the first isolate of a particular species per patient was accepted. All the strains were identified using ATB-Expression (biomérieux, France) and API automated systems (biomérieux, France), supplemented by conventional biochemical tests.

Antimicrobial susceptibility testing

The in vitro activities of antimicrobials were determined by the Kirby-Bauer Disk Diffusion Agar method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines[13]. The susceptibility of *Staphylococcus* to vancomycin and *Streptococcus pneumoniae* to penicillin and cefotaxime were determined by the Etest (MIC) method [13]. Yeasts and species with fewer than 30 isolates were not tested for antimicrobial susceptibilities. Quality control testing was performed by using *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S aureus* ATCC 25923 and *K. pneumoniae* ATCC 700603. All agar and disks were purchased from Oxoid (UK), and the Etests were supplied by biomérieux (France).

Screening tests for β-Lactamase production

β-Lactamase activity was determined by Nitrocefin-based test described by the CLSI [13].

Methicillin-resistant *Staphylococcus aureus* (MRSA) confirmation

The potential methicillin resistance in *S. aureus* isolates was screened for using the cefoxitin disk test described by the CLSI[13] and confirmed by PCR amplification of the mecA gene[14].

ESBL screening and confirmation

Screening for production of extended spectrum β-lactamases (ESBLs) by isolates of *E. coli, Klebsiella* spp., and *P. mirabilis* was performed as recommended by CLSI [13]. Confirmatory testing used the CLSI disk diffusion method with disks containing ceftazidime (30µg), ceftazidime-clavulanic acid (30µg/10µg), cefotaxime (30µg), and cefotaxime-clavulanic acid (30µg /10µg) supplied by Becton,Dickinsonand Company (USA).

VRE confirmation

Potential vancomycin resistance in *E. faecium* and *E. faecalis* (VRE) isolates was confirmed with the vancomycin agar dilution test by CLSI [13].

Statistical analysis

Data of antimicrobial susceptibility testing were entered into a standard format using WHONET 5.5 (WHO, Switzerland), which was used for data management. A P value of ≤ 0.05 was considered statistically significant. All statistical analysis was done by using SPSS 19.0 software (SPSS Inc., USA).

RESULTS AND DISCUSSION

In the surveillance study, pathogens isolated from an affiliated hospital of Shandong University in north China in one year were assessed. A total of 1641 isolates were characterized in different specimens from patients with infections diseases. The proportion of strains isolating from outpatients was merely 4.0%. Of these pathogens, 29.3% (480/1641) were gram positive strains and 70.7% (1161/1641) were gram negative strains, the difference were statistically significant ($P<0.001$). Table 1 showed the most common pathogens in the clinic, which were *E. coli* (20.5%), *P. aeruginosa* (18.1%), *S. aureus* (17.2%), *K. pneumoniae* (9.7%), *A. baumannii* (6.8%), and *Enterococcus spp.* (5.6%), *et al*. There were 4.0% (66/1641) strains isolated from outpatients and 96.0% (1575/1641) isolated from inpatients. These strains were cultured from respiratory samples (55.6%), followed by urine (20.0%), secretion (10.9%), blood (4.9%), CSF (1.6%) and others (7.0%). The top five strains according to isolation rates were *E. coli, P. aeruginosa, S. aureus, K. pneumoniae, and A.*

baumannii, respectively, which was nearly to the bacterial resistance surveillance data from Mohnarin and CHINET in 2011 in China. Nearly 55.6% of isolates recovered from clinical specimens were of respiratory origin. We recommend clinicians to pay more attention to the detection value of isolates in sterile fluids, so as to increase the examination rate of body fluids like blood, perfusate and serous effusion.

Table 2 summarized that resistance of gram-positive bacteria. Vancomycin was extremely effective, no vancomycin-resistant isolates were found in the surveillance study, which was similar to linezolid. 133 of 283 *S. aureus* isolates (47.0%) were found to be MRSA and 37 of 53 coagulase-negative *Staphylococcus* isolates(69.8%) were found to be MRCNS. It was obvious that the resistance rates of MRSA to most antibiotics were higher than MRCNS. MRSA was inactive to penicillin, oxacillin, erythromycin, rifampin, levofloxacin and gentamicin. Howevver, resistance rates of MRSA to chloramphenicol, SXT, minocycline and fusidic acid were lower, which were 11.3%, 4.7%, 3.9% and 3.8%, respectively. The resistance rates of MRCNS and MRSA to SXT were decreased from 63.9% to 4.7%, respectively, which were statistically significant (P<0.001). However, rifampin and gentamicin resistance rates increased from 2.7% to 76.5% and from 36.1% to 87%, respectively, for MRCNS and MRSA (P<0.001). Compared with the surveillance of foreign antibiotics resistance monitoring, the prevalence of MRSA was similar to that in India and Latin American countries, and higher than that in Europe (about 30%) and Oceania (about 20%) [15]. The exogenous *mecA* gene in MRS encoded amino acid residues 24 to 668 of penicillin-binding protein 2' (PBP2'), which showed low affinity to β-lactam antibiotics. Therefore, MRS could survive high dose of agents and showed resistance to multiple classes of antibiotics. Up to now, our hospital has not tested strains resistant to

Table 1. Most common pathogens isolated from patients in the JNCHSS 2012

Group	Pathogen	% (No.) of 1641 isolates	
Gram positive organism		29.3	(480)
	Staphylococcus aureus	17.2	(283)
	Enterococcus faecium	3.4	(55)
	Staphylococcus, coagulase negative[a]	3.3	(53)
	Enterococcus faecalis	2.2	(36)
	Streptococcus pneumoniae	2.0	(32)
	Streptococcus beta-haem.	0.9	(15)
	Streptococcus viridans, alpha-hem[a]	0.2	(4)
	Others	0.1	(2)
Gram negative organism		70.7	(1161)
	Escherichia coli	20.5	(337)
	Pseudomos aeruginosa	18.1	(297)
	Klebsiella pneumoniae	9.7	(159)
	Acinetobacter baumannii	6.8	(111)
	Stenotrophomos maltophilia	5.4	(89)
	Enterobacter cloacae	2.1	(35)
	Haemophilus influenzae	2.1	(35)
	Proteus mirabilis	1.5	(24)
	Morganella morganii ss. morganii	0.4	(6)
	Citrobacter freundii	0.3	(5)
	Enterobacter aerogenes	0.3	(5)
	Moraxella catarrhalis	0.3	(5)
	Serratia marcescens	0.2	(4)
	Other gram negative organism	3.0	(49)

[a] Clinical isolates from blood and sterile body fluids

vancomycin and linezolid yet, which can be used to cure severe infections with MRS. These findings will be helpful in assessing the appropriate empirical antibiotic regimen for infected with MRSA in order to shorten hospital stays and reduce costs [16].

The resistance rates of *E. faecium* to most antibiotics were higher than that of *E. faecasi*s. The resistence rates of *E. faecium* and *E. faecasi*s to ampicillin were 83.3% and 2.9%, respectively (P<0.001). However, the resistance rates of chloramphenicol and tetracycline to *E. faecium* and *E. faecasi*s, as exceptional antibiotics, increased from 2.3% to 48.5% and from 67.7% to 87.5%, respectively (*P*<0.001).

Infections with *Enterococcus* need co-treatment with two antibiotics, which can enhance killer effects: one was β-lactam or glycopeptides, the other was aminoglycosides. In our hostipal, sensitivities of *Enterococcus* to vancomycin and ticarcillin were both 100%.

All *S. pneumoniae* were isolated from patients with nonmeningitis, 43.7% of which (14/32) were children and 56.3% (18/32) were adults. The higher resistance rates to erythromycin, azithromycin, clindamycin and SXT were 96.9, 100, 78.2, and 75%. Only 2 of 32 isolates (6.2%) were PNSP (defined as having a penicillin MIC of ≥0.12 mg/L). No *S.pneumoniae* isolate was resistant to vancomycin or evofloxacin in the surveillance study. Our data suggested that these kinds of agents could not be used for treating community-acquired pneumonia (CAP). However, The concentration of azithromycin in lungs were much higher than that in serum, and it had satisfactory effects on atypical pathogen and *H. influenzae*, so it was still applied to cure infections in community. Azithromycin had better cooperate with β-lactam or fluoroquinolone antibiotics than using alone.

To Gram-negative bacteria (Table 3), *Enterobacteriaceae* strains were still very sensitive to carbapenem (imipenem and meropenem) antibiotics, except that two *K. pneumoniae* strains

Table 2. Antimicrobial resistance (%) to common antibiotics for the most common Gram-positive pathogens isolated from patients in the JNCHSS 2012

Antibiotics	*Staphylococcus aureus*		*Staphylococcus epidermidis*		*Enterococcus spp. pneumoniae*		*Streptoc-occus*
	MRSA N=133	MSSA N=150	MRCNS N=37	MSCNS N=16	*E. faecalis* N=36	*E.faecium* N=55	N=32
Penicillin	100	90.6	100	87.5	5.6	89.1	6.2 [c]
Oxacillin	100	0	100	0	-	-	-
Erythromycin	82.7	80.1	94.6	81.2	72.7[a]	95.2 [a]	96.9
Chloramphenicol	11.3	6.9	27	26.7	48.5 [a]	2.3 [a]	20.0
Clindamycin	72.0	66.5	75.7	37.5	-	-	78.2
Azithromycin	82.2	79.1	91.4	78.6	-	-	100
Trimethoprim-sulfamethoxazole	4.7	5.2	63.9	25	-	-	75.0
Rifampin	76.5	1.9	2.7	0	60.0	81.5	-
Linezolid	0	0	0	0	-	-	-
Levofloxacin	86.9	12	60	0	-	-	0
Cefoxitin	99.2	0.6	100	12.5	-	-	-
Gentamicin	87.0	39.7	36.1	20	-	-	-
Vancomycin	0	0	0	0	0	0	0
Fusidic acid	3.8	0	2.7	0	-	-	-
Minocycline	3.9	0.7	0	0	55.6	27.8	-
Ampicillin	-	-	-	-	2.9	83.3	-
Ticarcillin	-	-	-	-	0	0	-
Ciprofloxacin	-	-	-	-	50.0[b]	94.4 [b]	-
Tetracycline	-	-	-	-	87.5 [b]	67.7 [b]	-
Nitrofurantoin	-	-	-	-	0 [b]	11.4 [b]	-

[a] Not apply to urinary tract isolates. [b] Apply to urinary tract isolates only.
[c] reffer to Interpretive Standards for Penicillin parenteral (nonmeningitis)

Table 3. Antimicrobial resistance (%) to common antibiotics for the most common Gram-negative pathogens isolated from patients in the JNCHSS 2012

Antibiotics	*Escherichia coli*	*Klebsiella pneumoniae*N=159	*Enterobacter cloacae*N=35	*Pseudomonas aeruginosa*N=297	*Acinetobacter baumannii*	*Stenotrophomo-nas maltophilia* N=89	*Haemophilus influenzae* N=35
	N=337				N=111		
Ampicillin	90.0	-	-	-	-	-	48.6
Ciprofloxacin	72.1	29.3	5.7	20.2	46.8	-	0
Amikacin	10.7	7.5	2.9	12.1	45.9	-	-
Piperacillin	84	57.2	48.6	24.7	53.2	-	-
Piperacillin-tazobactam	9.5	13.2	23.5	22.3	47.7	-	-
Ticarcillin-clavulanicacid	60.4	52.2	37.1	40.0	51.8	-	-
Cefazolin	70.0	51	94.3	-	-	-	-
Cefotaxime	67.6	46.5	42.9	-	51.6	-	1.4
Ceftazidime	67.7	46.5	37.1	20.2	47.7	-	-
Aztreom	68.5	46.5	37.1	30.0	82.9	-	-
Cefepime	67.7	45.5	5.7	22.1	45.9	-	-
Imipenem	0	1.3	0	33.8	46.4	-	-
Meropenem	0	1.3	0	27.3	47.7	-	0
Cefuroxime	69.7	49.7	42.9	-	-	-	7.1
Cefoxitin	8.9	12.7	91.4	-	-	-	-
Nitrofurantoin [a]	10.2	42.1	-	-	-	-	-
Gentamicin	57.3	37.5	5.7	15.9	52.8	-	-
Levofloxacin	70.0	25.8	5.9	26.9	46.4	11.4	-
Trimethoprim-sulfamethoxazole	71.2	50.9	22.9	-	50.5	2.2	90.9
Minocycline	-	-	-	-	29.5	1.2	-
Chloramphenicol	-	-	-	-	-	-	17.1
Azithromycin	-	-	-	-	-	-	7.0

[a] Apply to urinary tract isolates only for *E. coli* was much higher ($P<0.001$).

were resistant to carbapenem antibiotics (the mechanism of resistance was carbapenemase-production). However, this advantage is not suitable for *P.aeruginosa* and *A.baumanni*i. *Enterobac- teriaceae* had a relatively low resistance against amikacin and piperacillin-tazobactam. ESBLs were produced in 60.5% of the *E.coli* strains, 44.7% of the *K. pneumoniae.* To *E. coli*, 72.1% and 70.0% of isolates were resistant to ciprofloxacin and Levofloxacin, respectively. In comparison, the rate of resistance to ciprofloxacin and levofloxacin were 29.3% and 25.8% for *K. pneumoniae* and 5.7% and 5.9% for *E. cloacae*. The rate of resistance to fluoroquinolone

The resistance mechanism of *Enterobacteriaceae* against β-lactam antibiotic was mainly through producing ESBLs. The emergence of ESBLs, in addition to high rates of fluoroquinolone resistance in all inpatient and outpatient Gram-negative isolates [17-18], has been identified by others as a cause for concern. In our study, the numbers of *E. coli* were the most. Second, third and fourth generation cephalosporins were found to be less active against *E. coli*, with moderate resistance rates ranging from 67.7% to 70.0%. Except for imipenem and meropenem, amikacin and piperacillin-tazobactam were effective against *E. coli*, with a low resistance rate of 10.7% and 9.5%. Resistance of *E. coli* to both fluoroquinolones was found to be high in the present study.

The resistance rates of *S. maltophilia* were 11.4, 1.2 and 2.2%, respectively, to levofloxacin, minocycline and SXT. Less than *30% of P. aeruginosa* strains were resistant to all antimicrobials except imipenem (33.8%) and ticarcillin-clavulanicacid (40.0%). *P. aeruginosa* has relatively high sensitivity to various antibiotics, but it is easy to plant to respiratory tract and form biological membrane to inhibit antibiotics infiltrating into bacteria. In his way, β-lactam can be expressed to induce drug-resistant mutation, produce Chromosome inducible enzyme and hydrolyze β-lactam antibiotics, which bring a lot of problems to cure infections.

More than *45% of A.baumannii* strains were resistant to all antimicrobials except minocycline (29.5%). *A. baumannii* is an opportunistic pathogen that is frequently involved in a variety of infections including pneumonia, septicaemia and urinary tract infection following hospitalization of patients with more severe illness [19]. The ability to cause outbreaks and chronically colonize patients which are usually hard to eradicate poses significant challenge to increases healthcare expenditure and infection control[20].

For 35 of *H. Influenzae* isolates, high susceptibility were remained to ciprofloxacin, cefotaxime, meropenem and cefuroxime, but the resistance rate to SXT was 90.9%. The incidence of β-lactamase production was 54.3%, which was higher than the data collected in China in 2000-2002 [21]. Nearly to 58% in South Korea and Taiwan[22-23].The average incidence of β-lactamase- producing of *H. influenzae* isolates in most European countries were about 20%, but is lower in some countries such as Italy (1.8%) [24]. The resistance patterns in our study approach to those of the South Korea and Taiwan. It may be the consequence of higher production of â-lactamase resulting from the pressure of a wider range of antibiotics. Average age of the studied patients may be another possible factor.

Our Surveillance of common clinical isolates showed that Gram-negative bacteria were the predominant pathogens and that antimicrobial resistance is severe in our hospital in north China, which may be related to illegitimate antibiotic use The increasing resistance rate of bacteria to a wide range of antibiotics leads to a serious clinical problem and threatens public health seriously. The severe situation appeals to apply to antibiotics reasonably and reinforce disinfection and isolation to reduce resistant strains, whose spread could be prevented and fallen down in this way. The result of the study can provide the basis for the rational usage of antimicrobial agents.

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