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Bacterial profiles and antibiotic resistance patterns in Xiangya Hospital, China

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The aim of this study was to characterize the bacterial profiles and antibiotic-resistance patterns in Xiangya Hospital in 2012, and provide guidance for rational use of antimicrobial agents. Clinical strains were identified by the Vitek 2 automatic microbe analysis system and API test strips, and minimal inhibitory concentrations (MICs) for each antibiotic agent was determined. Data were analyzed in the WHONET 5.4 software. 12,407 non-repetitive strains were identified in 2012, including 3,579 Gram-positive bacterial strains (28.85%), 7,579 Gram-negative bacterial strains (61.09%) and 1,249 fungi (10.06%). 53.63% Staphylococcus aureus are methicillin-resistant and 62.39% coagulase-negative Staphylococci are methicillin-resistant, but susceptible to vancomycin, teicoplanin or linezolid. Four Enterococcus faecium and 3 Enterococcus faecalis strains were resistant to vancomycin. 72.12% Escherichia coli and 56.23% Klebsiella pneumoniae were extended spectrum β-lactamases (ESBLs) positive, and carbapenem showed high activity against both bacteria (resistant rates <10%). Therefore, the number of bacterial pathogens isolated in this hospital and their antibiotic resistance situation were not optimistic. It is urgent and necessary to promote a wide, systematic, continuous and high-quality bacterial-resistance surveillance.

Key words: Antibiotic resistance pattern, bacterial profile, pathogen.

INTRODUCTION

According to a recent report, 80,000 deaths per year in China directly or indirectly resulted from antibiotics misuse, causing increases in bacterial resistance and an enormous damage to health (Yan et al., 2013). The irrational use of antibacterial agents includes incorrect selection of antibiotics, insufficient management of preventive medication and patients taking medicines without a doctor’s prescription, which was one of the foremost causes of antimicrobial resistance. Reports from various regions of China showed differences in bacterial profiles and antibiotic resistance patterns. Therefore, a long-term and continuous bacterial-resistance surveillance

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Abbreviations: MICs, Minimal inhibitory concentrations; ESBLs, extended spectrum β-lactamases; CLSI, Clinical and Laboratory Standards Institute; ISO, International Standardization Organization; CNS, coagulase negative staphylococci; MRSA, methicillin-resistant Staphylococcus aureus; MRCNS, methicillin-resistant coagulase negative Staphylococci; VRE, vancomycin-resistant Enterococcus.
program should be established and carried out, to understand the variation in local bacteria antibiotic resistance patterns and also guide in rational selection of antibiotics, and assist in developing relevant management measures of hospital infection control. This study characterized the profile of bacterial pathogens that were isolated in Xiangya Hospital in 2012, and analyzed the antibiotic resistance patterns.

MATERIALS AND METHODS

Bacterial strains

The specimens were collected from out-patients and in-patients who were undergoing a bacteriological examination in 2012 with bacterial infections. Pathogenic bacteria were cultured and isolated with appropriate media and environment. Concurrent quality control tests were performed by using the following standard strains: *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Enterobacter cloacae* ATCC 700323, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus aureus* ATCC29213 (all strains were provided by the National Center for Clinical Laboratories).

Identification

Clinical strains were identified using the Vitek 2 automatic microbe analysis system and API test strips (the identification system and API test strips were purchased from bioMerieux in France), then the minimal inhibitory concentrations (MICs) of antibiotic agents for each strain were tested by the Vitek 2 automatic microbe analysis system with its ancillary drug susceptibility cards (using broth microdilution method). The antibiotic susceptibility of a small number of the isolates was determined manually using the Kirby- Bauer method (drug slips were purchased from Oxoid Company in England). The antibacterial agents were tested following Clinical and Laboratory Standards Institute (CLSI) recommendations for antimicrobial sensibility tests, MRSA test, and extended spectrum β-lactamases (ESBLs) test.

Quality control

Xiangya Hospital is a well-known tertiary general hospital in China, the Department of Clinical Laboratory has acquired certification of International Standardization Organization (ISO) and all clinical microbiologists participating in this program have a laboratory qualification certificate and at least 3 years of work experience.

Data analysis

The results of the antimicrobial susceptibility tests were interpreted per CLSI standards. Data were analyzed using the WHONET 5.4 software. The same strain from the same type of specimen from one patient was counted once to avoid double counting of strain.

RESULTS

Bacterial profiles

A total of 12,407 non-repetitive strains were identified in 2012, including 3,579 (28.85%) Gram-positive bacterial strains, 7,579 (61.09%) Gram-negative bacterial strains and 1,249 (10.06%) fungi. The Gram-positive strains mostly consist of *S. aureus* (1,020 strains, 8.22%), coagulase negative *Staphylococci* (787 strains, 6.34%), *E. faecalis* (420 strains, 3.39%), *E. faecalis* (398 strains, 3.21%) and *S. intermedius* (87 strains, 0.70%). The main Gram-negative bacterial strains are *Acinetobacter baumannii* (1,524 strains, 12.28%), *Pseudomonas aeruginosa* (1,517 strains, 12.23%), *E. coli* (1,397 strains, 11.26%), *Klebsiella pneumoniae* (1,280 strains, 10.32%) and *Enterobacter cloacae* (352 strains, 2.84%) (Table 1).

Among the total 12,407 bacterial strains, 11,102 strains were isolated from in-patient's specimens, and the rest were from out-patients. The strains were isolated from respiratory tract specimens (sputum, throat, bronchial, broncho-alveolar lavage, etc.) (51.24%), genitourinary tract specimens (urine, prostatic fluid, vaginal secretions, etc.) (13.60%), wound secretions and pus (13.07%), paracentesis fluid (9.16%), blood and bone marrow (7.65%) and others (5.28%).

Antibiotic resistance patterns

**Staphylococcus**

For *S. aureus* or coagulase negative *Staphylococci* (CNS), methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant coagulase negative *staphylococci* (MRCNS) were 53.63 (547/1020) and 62.39% (491/787), but all strains were fully susceptible to vancomycin, teicoplanin or linezolid (Table 2). *S. aureus* was susceptible to doxycycline (81.76%) and chloramphenicol (79.61%), but strongly resistant (resistant rate over 60%) to penicillin, cefazidime, cefazolin, erythromycin and azithromycin (Table 2).

**Enterococcus**

*E. faecium* and *E. faecalis* were fully susceptible to teicoplanin and linezolid, but four isolates of *E. faecium* and three isolates of *E. faecalis* strains were resistant to vancomycin (Table 3). *E. faecium* were more resistant to almost all tested agents than *E. faecalis*, except tetracycline (Table 3). *E. faecalis* were highly susceptible to ampicillin (92.21%) and penicillin (84.92%) (Table 3).

**Enterobacteriaceae**

A majority of *E. coli* strains (72.12%) and *K. pneumoniae* strains (56.23%) were ESBLs positive, but carbapenem antibiotics are very effective, with resistant rates below 10% (Table 4). *E. coli* and *K. pneumoniae* were susceptible to the β-lactamase inhibitor compounds
### Table 1. Percentages of main strains (%).

| Bacterial pathogen                          | No.  | Percentage (%) |
|--------------------------------------------|------|----------------|
| *Acinetobacter baumannii*                  | 1,524| 12.28          |
| *Pseudomonas aeruginosa*                   | 1,517| 12.23          |
| *Escherichia coli*                         | 1,397| 11.26          |
| *Klebsiella pneumoniae*                    | 1,280| 10.32          |
| *Staphylococcus aureus*                    | 1,020| 8.22           |
| *Candida albicans*                         | 841  | 6.78           |
| Coagulase negative *staphylococci*         | 787  | 6.34           |
| *Enterococcus faecium*                     | 420  | 3.39           |
| *Enterococcus faecalis*                    | 398  | 3.21           |
| *Enterobacter cloacae*                     | 352  | 2.64           |
| *Stenotrophomonas maltophilia*             | 290  | 2.34           |
| *Smooth candida*                           | 225  | 1.81           |
| *Enterobacter aerogenes*                   | 202  | 1.63           |
| *Burkholderia cepacia*                     | 135  | 1.09           |
| *Serratia marcescens*                      | 117  | 0.95           |
| *Haemophilus influenzae*                   | 108  | 0.87           |
| *Proteus mirabilis*                        | 102  | 0.82           |
| *Streptococcus pneumoniae*                 | 87   | 0.70           |
| *Klebsiella oxytoca*                       | 74   | 0.60           |
| *Candida tropicalis*                       | 62   | 0.50           |
| *Streptococcus Feacalis*                   | 50   | 0.41           |
| *Streptococcus agalactiae*                 | 48   | 0.39           |
| Others                                     | 1,291| 10.40          |

### Table 2. Antibiotic resistance patterns of *Staphylococcus*.

| Antibacterial agents | *Staphylococcus aureus* | *coagulase negative staphylococci* |
|----------------------|-------------------------|------------------------------------|
|                      | S (%) | I (%) | R (%) | S (%) | I (%) | R (%) |
| Penicillin           | 3.14  | 0.00  | 96.86 | 10.29 | 0.00  | 89.71 |
| Oxacillin            | 46.37 | 0.00  | 53.63 | 37.61 | 0.00  | 62.39 |
| Cefazolin            | 31.67 | 1.66  | 66.67 | 35.96 | 4.07  | 59.97 |
| Ceftazidime          | 20.00 | 11.67 | 68.33 | 32.02 | 14.99 | 52.99 |
| Meropenem            | 37.55 | 4.31  | 58.14 | 54.13 | 8.39  | 37.48 |
| Vancomycin           | 100.00| 0.00  | 0.00  | 100.00| 0.00  | 0.00  |
| Teicoplanin          | 100.00| 0.00  | 0.00  | 100.00| 0.00  | 0.00  |
| Azithromycin         | 32.84 | 3.63  | 63.53 | 13.34 | 0.00  | 86.66 |
| Erythromycin         | 31.96 | 0.29  | 67.75 | 12.58 | 0.25  | 87.17 |
| Tetracycline         | 41.37 | 2.06  | 56.57 | 56.16 | 1.15  | 42.69 |
| Doxycycline          | 81.76 | 16.77 | 1.47  | 87.93 | 9.27  | 2.80  |
| Ciprofloxacin        | 47.65 | 1.96  | 50.39 | 44.85 | 9.66  | 45.49 |
| Levofloxacin         | 46.18 | 4.21  | 49.61 | 52.10 | 12.96 | 34.94 |
| Clindamycin          | 47.55 | 0.69  | 51.76 | 58.07 | 3.05  | 38.88 |
| Chloromycetin        | 79.61 | 12.35 | 8.04  | 77.38 | 1.27  | 21.35 |
| Rifampicin           | 55.69 | 2.45  | 41.86 | 79.29 | 0.38  | 20.33 |
| Linezolid            | 100.00| 0.00  | 0.00  | 100.00| 0.00  | 0.00  |

S, Susceptibility; I, insensitivity; R, resistance.
Table 3. Antibiotic resistance patterns of *Enterococcus faecium* and *Enterococcus faecalis*.

| Antibacterial agent | *Enterococcus faecium* | *Enterococcus faecalis* |
|---------------------|------------------------|------------------------|
|                     | S (%) | I (%) | R (%) | S (%) | I (%) | R (%) |
| Penicillin          | 14.29 | 0.00  | 85.71 | 84.92 | 0.00  | 15.08 |
| Ampicillin          | 7.86  | 0.00  | 92.14 | 92.21 | 0.00  | 7.79  |
| Vancomycin          | 95.00 | 4.05  | 0.95  | 96.98 | 2.27  | 0.75  |
| Teicoplanin         | 100.00| 0.00  | 0.00  | 100.00| 0.00  | 0.00  |
| Erythromycin        | 3.33  | 5.96  | 90.71 | 10.80 | 24.88 | 64.32 |
| Tetracycline        | 41.67 | 1.43  | 56.90 | 21.11 | 1.50  | 77.39 |
| Ciprofloxacin       | 14.05 | 3.81  | 82.14 | 67.59 | 10.55 | 21.86 |
| Levofloxacin        | 17.14 | 2.86  | 80.00 | 76.13 | 4.27  | 19.60 |
| Linezolid           | 100.00| 0.00  | 0.00  | 100.00| 0.00  | 0.00  |

S, Susceptibility; I, insensitivity; R, resistance.

Table 4. Antibiotic resistance patterns of *Escherichia coli* and *Klebsiella pneumoniae*.

| Antibacterial agent | *Escherichia coli* | *Klebsiella pneumoniae* |
|---------------------|--------------------|-------------------------|
|                     | S (%) | I (%) | R (%) | S (%) | I (%) | R (%) |
| Ampicillin          | 7.16  | 0.21  | 92.63 | 0.00  | 0.00  | 100.00|
| Ampicillin/Sulbactam| 24.27 | 24.91 | 50.82 | 35.86 | 14.14 | 50.00 |
| Piperacillin/Tazobactam | 88.69 | 6.44  | 4.87  | 79.22 | 8.05  | 12.73 |
| Cefazolin           | 20.54 | 0.00  | 79.46 | 26.25 | 11.80 | 61.95 |
| Cefepime            | 51.18 | 6.37  | 42.45 | 50.63 | 22.73 | 26.64 |
| Cefotaxime          | 30.99 | 6.95  | 62.06 | 27.50 | 13.12 | 59.38 |
| Ceftriaxone         | 25.34 | 11.24 | 63.42 | 46.88 | 0.39  | 52.73 |
| Cefotetan           | 93.99 | 1.72  | 4.29  | 91.72 | 2.11  | 6.17  |
| Ceftazidime         | 56.48 | 15.82 | 27.70 | 47.03 | 19.30 | 33.67 |
| Cefoperazone/Sulbactam | 92.56 | 6.29  | 1.15  | 86.72 | 7.19  | 6.09  |
| Cefuroxime          | 17.32 | 8.02  | 74.66 | 25.55 | 7.03  | 67.42 |
| Aztreonam           | 48.46 | 0.29  | 51.25 | 41.88 | 6.48  | 51.64 |
| Ertapenem           | 95.92 | 0.50  | 3.58  | 82.19 | 12.26 | 5.55  |
| Imipenem            | 98.07 | 0.21  | 1.72  | 95.63 | 2.18  | 2.19  |
| Meropenem           | 92.63 | 5.01  | 2.36  | 92.50 | 1.48  | 6.02  |
| Gentamicin          | 42.95 | 0.64  | 56.41 | 54.77 | 1.01  | 44.22 |
| Tobramycin          | 45.88 | 31.07 | 23.05 | 57.27 | 19.29 | 23.44 |
| Amikacin            | 89.69 | 5.51  | 4.80  | 86.64 | 0.78  | 12.58 |
| Levofloxacin        | 37.87 | 4.15  | 57.98 | 74.14 | 7.66  | 18.20 |
| Ciprofloxacin       | 35.86 | 1.65  | 62.49 | 67.50 | 5.62  | 26.88 |
| Trimethoprim/Sulfamethoxazole | 34.57 | 0.00  | 65.43 | 45.94 | 2.58  | 51.48 |

S, Susceptibility; I, insensitivity; R, resistance.

cefoperazone/sulbactam, but highly resistant to ampicillin (> 90%), and also resistant to cefazolin, cefoxime, cefotaxime and ceftriaxone (Table 4).

**Non-fermenters**

*A. baumannii* were resistant to ceftriaxone (78.99%), trimethoprim/sulfamethoxazole (76.63%), cefotaxime (72.75%) and ciprofloxacin (71.77%), but susceptible to amikacin (74.20%), minocycline (72.69%) and meropenem (53.84%) (Table 5). *P. aeruginosa* were susceptible (> 50%) to all tested antibacterial agents, and meropenem, amikacin and ciprofloxacin were the top three potential agents (Table 5).

**DISCUSSION**

Among the total 12,407 bacterial strains isolated in
Table 5. Antibiotic resistance patterns of *Acinetobacter baumannii* and *Pseudomonas aeruginosa*.

| Antibacterial agents               | *Acinetobacter baumannii* | *Pseudomonas aeruginosa* |
|-----------------------------------|---------------------------|--------------------------|
|                                   | S (%) | I (%) | R (%) | S (%) | I (%) | R (%) |
| Ampicillin/Sulbactam              | 41.37 | 10.17 | 48.46 |       |       |       |
| Piperacillin/Tazobactam           | 19.11 | 14.05 | 66.84 | 62.69 | 11.73 | 25.58 |
| Ceftazidime                       | 21.60 | 11.36 | 67.04 | 67.17 | 12.66 | 20.17 |
| Cefepime                          | 23.24 | 13.79 | 62.97 | 67.63 | 13.45 | 18.92 |
| Aztreonam                         | -     | -     | -     | 54.12 | 15.69 | 30.19 |
| Cefotaxime                        | 21.08 | 6.17  | 72.75 |       |       |       |
| Ceftriaxone                       | 8.27  | 12.74 | 78.99 |       |       |       |
| Imipenem                          | 29.81 | 1.58  | 68.61 | 65.46 | 6.86  | 27.69 |
| Meropenem                         | 53.84 | 25.67 | 20.49 | 65.46 | 6.86  | 27.69 |
| Gentamicin                        | 18.58 | 15.30 | 66.12 | 65.46 | 6.86  | 27.69 |
| Tobramycin                        | 31.58 | 3.29  | 65.13 | 67.11 | 6.13  | 26.76 |
| Amikacin                          | 74.20 | 1.51  | 24.29 | 75.81 | 3.75  | 20.44 |
| Minocycline                       | 72.69 | 27.31 | 0.00  |       |       |       |
| Ciprofloxacin                     | 23.90 | 4.33  | 71.77 | 68.16 | 5.14  | 26.70 |
| Levofloxacin                      | 38.61 | 29.54 | 31.85 | 62.69 | 13.18 | 24.13 |
| Ofloxacin                         | -     | -     | -     | 56.23 | 15.69 | 28.08 |
| Trimethoprim/Sulfamethoxazole     | 18.45 | 4.92  | 76.63 |       |       |       |

S, Susceptibility; I, insensitivity; R, resistance; -, no break point in CLSI.

Xiangya Hospital in 2012, there were more Gram-negative bacterial strains (61.09%) than Gram-positive ones (28.85%), and the top 5 pathogens were *A. baumannii*, *P. aeruginosa*, *E. coli*, *K. pneumoniae* and *S. aureus*, which composed of 54.31% of the total strains isolated and identified. The most common strains were *A. baumannii* (Gram-negative) and *S. aureus* (Gram-positive). *E. coli* (11.26%) had the highest relevance ratio in Enterobacteriaceae. The most frequent Non-fermenters was *Acinetobacter baumannii* (12.28%), followed by *P. aeruginosa* (12.23%). Of all the specimens, only 10.52% were collected from out-patients, suggesting that clinicians should be more aware of the importance of routine microbiological detections and apply appropriate tests for out-patients.

The relevance ratios of MRSA and MRSCN in 2012 were close to the results of last year, and the susceptibility rates of *S. aureus* and CNS to vancomycin, teicoplanin and linezolid also remained similar (Qun et al., 2011). MRSA had relatively high resistance rates to β-lactam antibiotics and may be resistant to many other antibiotics, leading to strong pathogenicity and high death rates. As compared to a national surveillance result, there is no obvious difference in the relevance ratio of MRSA (50.50%), and few strains were resistant to teicoplanin and linezolid (Yong-hong et al., 2012). According to a report from United States, an estimated 80,461 invasive MRSA infections occurred nationally in 2011, 48,353 were HACO infections, 14,156 were hospital-onset infections and 16,560 were community-associated infections (Dantes et al., 2013). Therefore, the burden of invasive MRSA infections was heavy.

Among clinical isolations of *Enterococcus faecium* and *Enterococcus faecalis* were the most prevalent ones. *E. faecium* displayed higher antibiotic resistance than *E. faecalis* (Qing et al., 2012; Sharifi et al., 2013). Our data shows a similar antibiotic resistance pattern, in which *E. faecalis* but not *Enterococcus faecium* were highly susceptible to ampicillin and penicillin. *E. faecium* were highly resistant to ciprofloxacin (82.14%) and levofloxacin (80.00%), which could be due to overuse of these antibiotics. We identified seven isolates of vancomycin-resistant *Enterococcus* (VRE), including four isolates of *E. faecium* (0.95%) and three isolates of *E. faecalis* (0.75%). The prevalence of VRE seemed more severe in India as shown by a report that 128 *Enterococcus* strains (2.30%) were isolated from a total of 5,555 clinical samples in one year (Sreeja et al., 2012). Among all the isolates, there were 97 isolates of *E. faecalis* (76%) and 31 isolates of *E. faecium* (24%).

For Enterobacteriaceae, our bacterial resistance data showed that they were susceptible to carbapenems (above 80%). Carbapenems were considered to be the most effective antimicrobial agents against Enterobacteriaceae infection, but there are more reports on Enterobacteriaceae resistance to carbapenems in China and other countries (Shi-guo, 2012; Castanheira, 2011) which should be noticed generally. *E. coli* and *K. pneumoniae* showed relatively high resistant rates to cefazolin, cefuroxime, cefotaxime and ceftriaxone, mainly due to ESBLs (Pitout and Laupland, 2008). From the results, 72.12% *E. coli* and 56.23% *K. pneumoniae* were
ESBL-positive, which were much higher than the ESBL-positive rate from other regions’ reports (9.7 and 12.7%; 13.51 and 16.55%) (Hawser et al., 2014; Chander and Shrestha, 2013). *E. coli* were intermediately resistant to ciprofloxacin (62.49%) and levofloxacin (57.98%), so clinicians should pay more attention to antibiotics selection when dealing with urinary system infection.

*A. baumannii, P. aeruginosa* and *S. maltophilia* were the most commonly identified Gram-negative non-fermenters in this hospital. These bacteria could survive all kinds of moisture environment in a hospital, naturally resistant to a variety of antibiotics and tend to develop into multi-drug resistant bacteria, which was quite a challenge for hospital infection control and clinical treatment. The analysis showed that the susceptibility rates of *A. baumannii* to most antibiotics were below 50%, except meropenem, amikacin and minocycline. *A. baumannii* were resistant to imipenem with a rate of 68.61%. A recent meta-analysis of carbapenem-resistant *A. baumannii* indicated the resistance mechanisms mainly contained carbapenemase production, outer membrane proteins and the Ade ABC efflux pump (De-song et al., 2013).

In summary, the number of bacterial pathogens isolated in Xiangya Hospital was very significant and the antibiotic resistance situation was not optimistic. It is urgent and necessary to promote a wide, systematic, continuous and high-quality bacterial-resistance surveillance. On the basis of this surveillance, clinicians should be more cautious when selecting and using antibiotics and the management of hospital infection control should be optimized. It is highly recommended, in order to avoid the bacteria resistance increasing and prevent the new antibiotic-resistant strains, to strictly control and rationally use antibiotics, enhance the overall effects on hospital infection control measures, and pays more attention to hospital disinfection and isolation.

### Conflict of Interests

The author(s) have not declared any conflict of interests.

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