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Major Article

Antimicrobial resistance of 3 types of gram-negative bacteria isolated from hospital surfaces and the hands of health care workers

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Key Words:
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Background: There has been an increased focus in recent years on antimicrobial resistance of bacteria isolated from clinical samples. However, resistance of bacteria from hospital environments has been less frequently investigated.

Methods: According to hygienic standard for disinfection in hospitals, samples were collected from hospital inanimate surfaces and the hands of health care workers after daily cleaning. An automatic microorganism analyzer was used to identify bacteria and test for antimicrobial susceptibility. Polymerase chain reaction was used to detect antimicrobial resistance genes.

Results: The detection rate of bacteria in general wards was significantly higher than that in intensive care units. The isolates were predominantly gram-negative (GN) bacteria, with *Pseudomonas aeruginosa*, *Enterobacter cloacae*, and *Klebsiella pneumoniae* being the most common. *P aeruginosa* isolates from other surfaces were much higher than those from medical instruments. *E cloacae* was isolated more frequently from the hands of other staff than medical staff. Most *P aeruginosa* and *K pneumoniae* were resistant to sulfonamides and β-lactam antimicrobials. Only 1 strain of *P aeruginosa* and 1 strain of *K pneumoniae* showed multiple antimicrobials resistance.

Conclusions: The GN bacteria isolated from hospital environments demonstrate variable resistance to antimicrobials.

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In recent years, there has been a large number of studies on the antibiotic resistance of clinical isolates.[1,2] However, the antibiotic resistance of isolates from hospital environments has received less attention. Previous studies have shown that a variety of bacteria may persist on environmental surfaces in hospitals and on the hands of health care workers (HCWs), even after cleaning and disinfection.[3,4] These bacteria can easily be transferred from hospital surfaces to the hands of HCWs, and then be spread to vulnerable patients elsewhere in the hospital.[5,6] Physicians may encounter considerable difficulty treating patients infected by these pathogenic bacteria which often harbor antimicrobial-resistance mutations.[7-9] This study aimed to explore the antimicrobial resistance of 3 types of gram-negative (GN) bacteria isolated from hospital surfaces and the hands of HCWs. We hope to provide a scientific basis for improving the efficacy of disinfection and strengthening infection control and prevention measures aimed at reducing health care–associated infections (HAIs).

MATERIALS AND METHODS

Source of samples

Bacteria were isolated from common inanimate surfaces and the hands of HCWs (eg, medical staff, others) in the intensive care units (ICUs) and general wards of 16 hospitals (6 secondary hospitals and 10 tertiary hospitals) in Beijing. The inanimate surfaces included hospital bed units (pillows, pillowcases, pillow towels, bedsheets, bed rails, etc), medical instruments (stethoscopes, blood pressure cuffs, etc), and other surfaces (water taps, thermos bottles, treatment carts, dishcloths, etc). Three standard strains, including *Pseudomonas aeruginosa* (ATCC 154426; ATCC, Rockville, MD), *Escherichia coli* (ATCC 13706; ATCC), and *Klebsiella pneumoniae* (ATCC 27336; ATCC) were used as the quality control strains for antimicrobial susceptibility testing and genetic testing.

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Sample collection

In adherence with hygienic standard for disinfection in hospitals (GB 15982-2012),[10] samples were collected from hospital inanimate surfaces and the hands of HCWs in ICUs and general wards of 16 hospitals (6 secondary hospitals and 10 tertiary hospitals) in Beijing. After daily cleaning, a sterile cotton swab moistened with sterile saline solution was moved over inanimate surfaces 5 times in the transverse and longitudinal directions, while concurrently turning the cotton swab. For larger surfaces, 100 cm^2 were swabbed, and for surfaces below this cutoff, the entire surface was sampled. For sample collection from the hands of HCWs, a sterile cotton swab moistened with sterile saline solution was moved twice over the flexor surfaces of the fingers on both hands from the base of the fingers to fingertips (the area of one hand was approximately 30 cm^2), while concurrently turning the cotton swab. The portion in contact with the collector's hand was removed, and the remaining portion was placed into a test tube containing 10 mL sterile saline solution. Informed consent was obtained from all HCWs. The study was approved by the Human Ethics Committee of Beijing Chaoyang District Center for Disease Control and Prevention.

Isolation, identification, and antimicrobial susceptibility testing

Isolation and culture of pathogenic bacteria were conducted in accordance with the manual of clinical microbiology. An automatic microorganism analyzer (VITEK2 COMPACT; bioMérieux, Marcy-l'Étoile, France) was used to identify bacteria and antimicrobial susceptibility. AST-GN09 cards (bioMérieux) were used to assess antimicrobial susceptibility of *P aeruginosa*, *Enterobacter cloacae*, and *K pneumoniae* to 21 different antimicrobials, including ampicillin (AM), ampicillin-sulbactam (SAM), piperacillin (PIP), piperacillin-tazobactam (TZP), cefazolin (CZ), cefuroxime (CXM), cefuroxime axetil (CEFTIN), cefotetan (CTT), ceftazidime (CAZ), ceftriaxone (CRO), cefepime (FEP), aztreonam (ATM), imipenem (IPM), meropenem (MEM), amikacin (AN), gentamicin (GM), tobramycin (TM), ciprofloxacin (CIP), levofloxacin (LEV), nitrofurantoin (FT), and trimethoprim-sulfamethoxazole (SXT). Evaluation of antimicrobial susceptibility results was performed in accordance with the Clinical and Laboratory Standards Institute.[10]

Detection of drug resistance genes

DNA extraction was performed using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). The DNA template was stored at –20°C. Primer Premier 6.0 software (Premier Company, Alberta, Canada) was used to design primers for the target genes associated with sulfanilamide groups (*sul1*/*sul2*), β-lactam antimicrobials (*tem-1*, *shv*, *dha*, *vim*, and *mir*), and aminoglycosides [*aph(3')-I*]. Polymerase chain reaction (PCR) primer sequences and product lengths of target genes are listed in Table 1. DNA amplification was initiated by incubating the sample for 5 minutes at 96°C. Then the samples underwent 30 cycles of denaturation, annealing, and synthesis. After all 30 cycles, the sample was kept at 72°C for 8 minutes. PCR products were resolved on a 2.0% agarose gel, and the bands were visualized with ethidium bromide. Gene sequencing was performed by Beijing Jimei Biotechnology.

Statistical analysis

The proportions of different groups were compared by χ^2 analysis using SPSS 22.0 (IBM company, Armonk, NY). A P value of <.05 was considered statistically significant.

Table 1
Polymerase chain reaction primer sequences and product lengths of target genes

Target genes	Primer sequences	Product lengths (bp)
sul1	F: 5′-GTGACGGTGTTCGGCATTCT-3′ R: 5′-TCCGAGAAGGTGATTGCGCT-3′	779
sul2	F: 5′-TTCGGCATCGTCAACATAACCT-3′ R: 5′-CGTGTGTGCGGATGAAGTCAG-3′	727
tem-1	F: 5′-AGGAAGAGTATGATTCAACA-3′ R: 5′-CTCGTGGTTTGGTATGGC-3′	535
shv	F: 5′-GGTTATGCGTTATATTCGCC-3′ R: 5′-TCCCGCAGATAAATCACCA-3′	786
dha	F: 5′-AACTTTCACAGGTGTGCTGGGT-3′ R: 5′-CCGTACGCATACTGGCTTAGC-3′	405
vim	F: 5′-ATTCCGGTCGG(A/G)GAGGTCCG-3′ R: 5′-GAGCAAGTCTAGACCGCCCG-3′	633
mir	F: 5′-TCGGTAAAGCCGATGTTGCGG-3′ R: 5′-CTTCCACTGCGGCTGCCAGTT-3′	302
aph(3')-I	F: 5′-ATGTGCCATATTCAACGGGAAACG-3′ R: 5′-TCAGAAAAACTCATCGAGCATCAA-3′	816

F, forward primer; *R*, reverse primer.

RESULTS

Types and distribution of pathogenic bacteria

A total of 979 samples were collected from hospital surfaces (868 samples) and the hands of HCWs (111 samples) at 16 hospitals. The overall bacterial detection rate was 7.66%. There was no difference in the detection rates of bacteria between the secondary and tertiary hospitals. However, the detection rate of bacteria in general wards was significantly higher than that in ICUs ($\chi^2 = 13.40$, $P < .01$), which was 10.83% and 4.61%, respectively (Table 2).

Table 3 shows that 65 strains of bacteria were isolated from hospital surfaces (868 samples), yielding a detection rate of 7.49%. There was a significant difference in the detection rate of bacteria on the surfaces of hospital bed units, medical instruments, and other objects ($\chi^2 = 6.95$, $P < .05$). Furthermore, the detection rate of bacteria on the surface of other objects was significantly higher than that of medical instruments ($\chi^2 = 7.00$, $P < .01$), which was 9.54% and 2.91%, respectively. Also, 10 strains of pathogenic bacteria were isolated from the hands of HCWs (111 samples), with a detection rate of 9.01%. The detection rate of pathogenic bacteria from other staff (21.21%) was significantly higher than that from medical staff (3.85%; $\chi^2 = 6.36$, $P < .05$). The isolates were predominately GN bacteria, with *P aeruginosa* (24 strains), *E cloacae* (14 strains), and *K pneumoniae* (four strains) being the 3 most common. Additional strains of GN bacteria included *E coli*, *Serratia rubidaea*, *Serratia marcescens*, *Stenotrophomonas maltophilia*, *Klebsiella oxytoca*, *Acinetobacter baumannii*, and others. Among all 24 strains of *P aeruginosa*, 12 strains were isolated from the surfaces of hospital bed units, 9 strains were isolated from other surfaces, and 3 strains were isolated from the hands of HCWs. There was a significant difference in the detection rate of *P aeruginosa* on surfaces of different objects ($\chi^2 = 6.12$, $P < .05$). Furthermore, the detection rate of *P aeruginosa* on the surface of other objects was significantly higher than that of medical instruments ($\chi^2 = 6.57$, $P < .01$). As for *E cloacae*, 7 strains were isolated from the surfaces of hospital bed units, 5 strains were isolated from other surfaces, and 2 strains were isolated from the hands of other staff. No significant difference was found in the detection rate of *E cloacae* on the surfaces of different objects. However, the detection rate of *E cloacae* on the hands of other staff (6.06%) was significantly higher than that of medical staff (no isolates were found) ($\chi^2 = 4.81$, $P < .05$). Four strains of *K pneumoniae* were isolated from a blood pressure cuff, a stethoscope, a water tap, and an isolation gown. The detection rate of *K pneumoniae* on surfaces of medical

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