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Multi-Drug Resistance in Health Care-Associated Bacteremia in Intensive Care Units at King Fahad Specialized Hospital, Buraidah, Saudi Arabia

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Abstract Health care-associated bacteremia cause important morbidity and mortality and have a considerable impact on healthcare costs. Infections caused by extended spectrum beta-lactamase producing bacteria are of clinical and epidemiological importance, since their mobile genetic elements facilitate cross-infection. Objectives: To analyze health care-associated bacteremia in ICUs of King Fahad Specialized Hospital and to assess ESBL production in the isolated Gram negative bacteria. Methods: This study included 519 patients. Their blood samples were collected for blood culture. The isolates were identified and antibiotic sensitivity tests were performed. The type of B-lactamase gene was determined by polymerase chain reaction (PCR). Results: The rate of health care-associated bacteremia was 9.8%. Gram positive organisms were detected in 67.8 %; methithilin resistant *Staph.aureus* (MRSA) was the most prevalent (17.8 %). Gram negative bacilli were detected in 30.6 %. *E.coli* was the most common (12.9%). The production of extended spectrum β-lactamase (ESBL) enzyme was positive in 84.2% of the isolated Gram negative isolates. Temoniera (TEM) was the main type of β-lactamase. Conclusion: The isolation of multi-drug resistant bacteria and ESBL producing Gram negative organisms in ICU patients resulted in a greater awareness of implementation of new rules for microbiological screening and infection control measures.

Keywords Health Care-Associated Bacteremia, *Klebsiella Pneumoniae,* Methicillin Resistant *Staph Aureus,* Extended Spectrum Beta-lactamase

1. Introduction

Health care-associated infections (HAI) cause important morbidity and mortality and have a considerable impact on healthcare costs. Effective infection control programs, such as surveillance, can reduce the infection rate by up to 32%[**1**].

Health care-associated blood-stream infections (BSI) create a serious health problem in hospitals all over the world. In addition, patients admitted to intensive care units (ICUs) carry an even higher risk of BSI than those admitted to other types of units (non- ICU) and data from the surveillance and control of pathogens of epidemiologic importance in United States hospitals showed that 49.4% of all BSI occurred in the ICU[2]. HAI are frequently associated with drug-resistant microorganisms, including methicillin resistant *Staphylococcus aureus* (MRSA) and extended spectrum β-lactamase (ESBL)-producing Gram negative bacteria, which can pose considerable therapeutic problems[3].

Antimicrobial resistance is an increasing threat in hospitalized patients, and both mortality and morbidity from infections are greater when caused by antimicrobial-resistant bacteria[4]. It is also noted that there is an increase in the resistance among Gram negative bacilli to third generation cephalosporins which is caused by expression of ESBL enzymes.

ESBLs are enzymes that mediate resistance to extended-spectrum (third generation) cephalosporins (e.g., ceftazidime, cefotaxime, and ceftriaxone) and monobactams (e.g., aztreonam) but do not affect cephamycins (e.g., cefoxitin and cefotetan) or carbapenems (e.g., meropenem or imipenem)[5-7].

The aim of the present study is to identify health care-associated bacteremia in the ICUs and to describe the resistance patterns of the isolates especially those caused by ESBLs. The study also aimed to determine the types of β-lactamase genes responsible for resistance among *K pneumoniae* isolates by polymerase chain reaction because *K pneumoniae* bacteremia is a very important cause of morbidity and mortality in Gram negative bacteremia in

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most medically well-developed countries.

2. Methodology

2.1. Study Design and Subjects

The present study was conducted over a period of 12 months from May 2011 to April 2012 on patients admitted to different ICUs at KFSH. The total number of patients included in the study was 519 patients. They were monitored daily by the attending physicians for subsequent development of health care-associated bacteremia.

2.2. Sample Collection and Processing

Blood samples for culture were collected before the start of antibiotic therapy. The samples were inoculated immediately under complete aseptic conditions into bottles containing 50ml brain heart infusion broth. The bottles were incubated aerobically at 37℃ for 7 days and were examined daily for evidence of bacterial growth. Subcultures were done on blood agar.

2.3. Identification of Bacterial Isolates

Isolated colonies were evaluated according to their morphology, motility, Gram staining characteristics, catalase oxidase, coagulase tests, PYR test and latex agglutination test for Streptococci identification (Omega Diagnostica, Scotland, UK). Gram negative bacilli were further identified by API 20E system (Bomeriux SA, Monlien Vercica and France). Wet mount preparations were used to determine Candida species which was identified by germ tube test.

2.4. Detection of Oxacillin Resistance

Detection of oxacillin resistance was made by using Oxacillin Resistant Screening Agar Base[8].

2.5. Antimicrobial Susceptibility

Susceptibility was tested by modified Kirby-Bauer disc diffusion method using 0.5 McFarland turbidity of each isolate[**9**].

2.6. Detection of β-Lactamase Production

Beta-lactamase enzyme is detected among the Gram negative bacilli by the chromogenic method (Nitrocefin test- Oxoid, UK).

2.7. Phenotypic Detection of Extended Spectrum β-Lactamases

This was done by the combined disc method and E-test using Cefotaxime/cefotaxime + clavulanic acid (CT/ CTL) and Ceftazidime/ceftazidime + clavulanic acid (TZ/TZL) (AB BIODISK, Solna, Sweden)[10].

2.8. Genotypic Detection and Determination of the Type of β-Lactamase

Isolated strains of *Klebsiella pneumoniae* were investigated to determine the probable type of β-lactamase enzyme. The isolates were tested for TEM, SHV, CTX-M-1 and TOHO-1 genes by using PCR. DNA was extracted from clinical isolates and controls according to the method described by[11]. PCR reaction was performed in a final volume of 50µl containing 0.2 µM of each primer, 200µM of each dNTPs, 1x reaction buffer, 2.5 units of Taq polymerase and 5 µM of the template DNA. Klebsiella strains with known ESBL types: TEM, SHV, CTX-M-1 and TOHO-1were included as positive controls. A reagent blank containing all components of the reaction mixture except template DNA (substituted with sterile distilled water) was included in PCR reaction as a negative control. The amplification conditions were as follows: 94℃ for 5 min; then 35 cycles at 94℃ for 30 s, 58℃ for 30 s, and 72℃ for 2 min; and, finally, one cycle at 72 ℃ for 15 min.**[12-14].** Primers sequences for are shown in table (1).

Table 1. Primer Sequences for Detection of β–Lactamases Genes

Gene	Primers sequences	Product size (bp)
CTXM-1	F:5'-GACGATGTCACTGGCTGAGC-3' R:5'-AGCCGCCGACGCTAATACA-3'	499
TEM	F:5'-TTGGGTGCACGAGTGGGTTA-3' R:5'-TAATTGTTGCCGGGAAGCTA-3'	503
SHV	F:5'-TCGGGCCGCGTAGGCATGAT-3' R:5'-AGCAGGGCGACAATCCCGCG-3'	625
TOHO-1	F:5'-GCGACCTGGTTAACTACAATCC-3' R:5'-CGGTAGTATTGCCCTTAAGCC-3'	351

4. Results

A total of 51 patients developed health care-associated BSI (9.8 %). The age of the patients ranged between 5 and 75 years with a mean age of 42.4 ± 16.7 years. Number of males was 36 out of 51 (70.6%)

The rate of bacteremia among different intensive care units (ICUs) is illustrated in table (2) which shows that the highest rate was in the chest ICU (19.5%) followed by the trauma care unit (12.5%) then the general ICU (9.9%).

Out of 51 BSI patients, 45 patients had monomicrobial infection (88%) and 6 had polymicrobial infection (12%). Among these infections, 62 different microorganisms were isolated. Analysis of these microorganism showed that Gram positive bacteria were reported in 42 isolates (67.8 %) while Gram negative bacteria were reported in 19 isolates (30.6%). MRSA was isolated from 11/62 (17.8%), followed by methicillin resistant coagulase negative Staphylococci 10 (16%), *E coli* 8 (12.9%), *Klebsiella pneumoniae 6 (9.7%), Candida spp.* in one isolate (1.6%) (Table 3).

Antibiotic sensitivity tests of Gram positive organisms showed that the isolated organisms were mostly sensitive to vancomycin (85.7%), amikacin (47.6%) and chloramphenicol (42.9%). While, Gram negative organisms were mostly sensitive to imipenem (89.4%), amikacin (42.1%) and gentamicin (31.5%).

Table 2. Bacteremia among Different ICUs

ICU	Frequence	Rate	Proportion
Trauma (n=80)	10	12.5	19.6
Post Operative (n=26)	2	7.7	3.9
Chest (n=87)	17	19.5	33.3
Medicine (n=92)	3	3.3	5.9
General (n=81)	8	9.9	15.7
Coronary (n=83)	6	7.2	11.8
Tropical (n=70)	5	7.1	9.8
Total (n=519)	51	9.8	100.0

*(Rate is calculated according to the number of patients admitted to each ICU)
**(Proportion is calculated according to the total number of infected patients)

Table 3. Distribution of Pathogens Associated with NBSI

Pathogens	No (%) n=62
Gram-Positive Organisms	42 (67.8)
MRSA	11
MRCoNS	10
MSSA	8
S.pneuminae	5
Enterococcus spp.	5
Viridans streptococci	3
Gram–Negative Organisms	19(30.6)
E.coli	8
K.pneumoniae	6
Enterobacter species	2
Acinetobacter baumannii	1
Pseudomonas aeruginosa	2
Candida spp.	1(1.6)

3.1. Analysis of β-lactamase producers

Out of 62 organisms causing BSI, 19 (30.6%) were Gram negative bacilli (table 4). All these strains were screened for the production of β-lactamase enzyme by nitrocefin test, which indicated that 16/19 (84.2%) were β-lactamase producers with the largest percent (100%) being in *E coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

Table 4. Gram Negative Bacilli Isolated from BSI and β-Lactamase Production

Microorganism	No (%) n=19	β-Lactamse Production
E coli	8 (42.1%)	8(100%)
Klebsiella pneumoniae	6(31.5%)	4(66.7%)
Enterobacter spp.	2(10.5%)	1(50%)
Acinetobacter baumannii	1(5.4%)	1(100%)
Pseudomonas aeruginosa	2 (10.5%)	2(100%)
Total Gram Negative Bacilli	19	16/19(84.2%)

3.2. Analysis of ESBL production by confirmatory tests among Gram negative bacilli

Confirmatory tests for ESBL production including Combined Disk method and ESBL E-Test revealed that 12/19 (63.2%) of isolates was confirmed to be ESBL producers (Table 5). ESBL production was confirmed in 6/8 *E coli* strains (75%), 4/6 *K pneumoniae* (66.7%), 1/2 *Enterobacter species* (50%) and 1/1 *Acinetobacter baumanii* (100%).

3.3. Determination of the type of β-lactamase by PCR in *K pneumoniae* isolates

Types of β-lactamase results are shown in table (6). It can be noticed that TEM was the main type of β-lactamase, SHV was the second, followed by CTX-M1, no TOHO-1 genes were detected in the tested isolates.

Table 5. Preliminary screening and confirmatory tests for ESBL among Gram negative organisms isolated from patients

Organisms (n=19)	Screening	Confirmatory tests	
		Combined disk method	ESBL-E Test
E coli 8	8(100%)	6(75%)	6(75%)
K pneumoniae 6	4(66.7%)	4(66.7%)	4(66.7%)
*Enterobacter spp.*2	1(50%)	1(50%)	1(50%)
Acinetobacter baumannii 1	1(100%)	1(100%)	1(100%)
Pseudomonas aeruginosa 2	2(100%)	0(0%)	0(0%)
Total 19(100%)	16(84.2%)	12(63.2%)	12(63.2%)

Table 6. Distribution of β-Lactamase Genes among *K pneumoniae* strains

Gene Type	*Klebsiella pneumoniae* (n=6)
TEM	5(83.3%)
SHV	4 (66.7%)
CTX-M-1	3(50%)
TOHO-1	0 (0%)
TEM+SHV	3(50%)
TEM+CTX-M-1	3(50%)
TEM+SHV+CTX-M-1	1(16.7%)

3.4. ESBL Production by Phenotypic Methods and Correlation with PCR

Out of 6 isolates of *K pneumoniae* strains, 5 strains (83.3%) could be identified as ESBL producers by PCR, 4 of them (66.7%) could be identified as ESBL producers by Combined Disk method and ESBL E-Test, the strain which was negative by phenotypic methods showed TEM enzyme by PCR.

4. Discussion

NBSI create a serious health problem in hospitals all over the world. They contribute to greater morbidity and mortality rates, as well as to increasing length of hospital stay and health care cost[3]. In the present study, the rate of health care-associated BSI was 9.8%. This result was higher than those reported by[15] in Canada (0.69%).

The high rate of health care associated BSI in this study may be due to the high risk patients enrolled in the study and the presence of several risk factors associated with those patients.

In this study we tried to assess the risk factors associated with BSI. There was a higher significant difference in patients with previous prescription of antibiotics who developed BSI (34/51, 66.7%) compared to 216/468 of patients without BSI (46.2%) (P = 0.02), there was also a higher significant difference in patients on mechanical ventilation who developed BSI (18/51, 35.3%) compared to 102/468 of patients without BSI (21.8%) (P = 0.03). There was also higher percentage of patients with urinary catheterization who developed BSI 20/51 (39.2%) compared to 129/468 of patients who had no BSI (27.6%), but with non-significant difference (P = 0.081), This is supported by the results of another study**[2]** who reported that risk factors for acquisition of BSI in the ICU included previous prescription of antibiotics, mechanical ventilation and the use of nasogastric tube.

Our results were in concordance with surveillance for nosocomial bloodstream infections at 49 hospitals over a 3-year period detected >10,000 infections. Gram positive organisms accounted for 64% of cases, Gram negative organisms accounted for 27%. The most common organisms were coagulase negative staphylococci (32%), *Staphylococcus aureus* (16%)[16].

In our study, we noticed that MRSA strains had high overall resistance to penicillin (100%), erythromycin (90.9%), clindamycin (81.8%), ampicillin (100%), ciprofloxacin (72.7%), tetracycline (63.6%), cefotaxime (81.8%), gentamicin (63.6%) and amoxicillin/clavulanic (90.9%). These results agreed with[8], who found that resistance rates of MRSA were as follows: ciprofloxacin (89%), clindamycin and erythromycin (94% for each), tetracycline (33%).

In our study, few isolates (9.1%) were found to be vancomycin resistant. This report paralleled what is reported by different studies which indicated that vancomycin intermediate *S. aureus* strains are beginning to emerge[4].

IN the present study, Gram negative organisms were mostly sensitive to imipenem (89.4%), amikacin (42.1%) and gentamicin (31.5%), these results are similar to[17] who reported 47.7% resistance to amikacin and 81% resistance to gentamycin.

***Kanamori et al*[18]** showed that 71.4% of the isolates were resistant to gentamycin, while all of the ESBL-producing isolates were susceptible to imepenem. It has been reported that the prevalence of coresistance to CIP, GEN, and SXT was high among phenotypically identified ESBL-producing E. coli and K. pneumoniae in the Philippines[19]. Imipenem remains fully effective against ESBL producers and carbapenems may be the most appropriate agents for severe infections due to ESBL-producing Enterobacteriaceae[14],[17-18].

In the present study, a total of 63.2% (12/19) of isolates were confirmed to be ESBL producers. These results are in concordance to[14] who found 73.2% ESBL phenotypic expression among Enterobacteria. ***Akujobi and Ewuru Chika et al*[9]** reported ESBL production in 11.4% of Klebsiella isolates. A study confirmed ESBL production in 17/91 (18.7%) of strains of Enterobacteriacae and among all of the 3 klebsiella species tested[18]. Another study reported ESBL production in 65/207 (31.4%) of Klebsiella, Enterobacter and Serratia strains with a predominance of Klebsiella pneumoniae (29/65)[19]. The resistance phenomenon is on the increase, this increasing resistance is due to inappropriate usage of antimicrobial drugs such as overuse, misuse, suboptimal dosage and non-compliance with treatment duration[9].

In the present study, the type of β-lactamase gene was determined among *K pneumoniae* strains by PCR in which TEM was the main type of β-lactamase, followed by SHV, then CTX-M1. Some *K pneumoniae* strains produced more than one type of β-lactamase. *Vasques et al*[14] reported TEM in 13/24, CTX-M1 in 7/24 and SHV in 6/24 of ESBL producing Enterobacteria and they also reported that Klebsiella and E coli predominated in the ESBL producing species identified. Predominance of SHV in 13/14 of *K pneumoniae* strains followed by TEM and CTX-M (9/14 for each of them) was reported[17]. Several studies showed expression of more than one ESBL genes which is in concordance to the present study[14],[17],[20].

5. Conclusions

The isolation of multi-drug resistant bacteria and ESBL producing Gram negative organisms in ICU patients during the study period resulted in a greater awareness to the presence of multi-drug resistance among bacterial isolates in our hospital ICU units. New rules for microbiological screening and infection control measures are recommended i.e. testing for beta-lactamase production and isolation precautions for patients in whom these microorganisms are identified. In addition, restriction of the use of

third-generation cephalosporins, along with care in the use and abuse of antimicrobials to minimize the emergence of resistant strains, are the most effective strategies for controlling and decreasing blood stream infections and the spread of ESBL-producing and multi-drug resistant organisms.

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