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Surveillance of Antibiotic-Resistant Bacteria in King Khalid Hospital, Hafr Al-Batin, Saudi Arabia, During 2013

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Abstract

Background: Research to understand and control the emergence and spread of antimicrobial resistance has become a public health priority.
Objectives: This study was conducted to study epidemiology and resistant pattern of bacteria causing infection in different King Khalid hospital units.
Patients and Methods: All samples were sent to the lab and routinely processed according to the standard microbiological procedures. Then, the cultures yielding growth were selected for the study. Identification and antibiotic susceptibility test for all clinical isolates were processed by using MicroScan instrumentation. A total of 428 clinical samples were collected within 8 months; out of them, 300 clinical isolates were subjected to validation test.
Results: *Escherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa* were the commonly identified Gram-negative bacteria. *Staphylococcus aureus* was the only identified Gram-positive bacterium. The most common infections were taken from the wounds (39.0%), urinary tract (32.3%), and bloodstream (17.8%). The most common antibiotic-resistant bacteria were found on female surgical ward (100%) followed by ICU (90.2%), and male surgical ward (88.2%). The overall results of antibiotic resistance were 100% for *S. aureus*, 93.3% *K. pneumonia*, 75.7 % *E. coli*, and 100% for *P. aeruginosa*. *Staphylococcus aureus* showed high resistance to ampicillin and linezolid (94.1%). High (86.95%) and full resistance (100%) against ampicillin were observed from *E. coli* and *K. pneumonia*, respectively. *P. aeruginosa* was fully resistant to 4 antibiotics of cefazoline, cefoxitin, tetracycline, and trimethoprim-sulfamethoxazole.
Conclusions: The study was useful in determining the risk factors and defining different hospital units which should be targeted for measures to prevent infection.

Keywords: Resistance, Clinical Wards, *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*

1. Background

Antibiotic resistance is one of the most important global health problems, which leads to increased morbidity, mortality, and health care costs. Distribution of resistance pathogens to antibiotics differs and depends on time, hospital, and different hospital wards (1). Overall, the excessive use of antibiotics leads to the emergence of antimicrobial resistance; therefore, the rational use of antibiotics leads to control the spread of this resistance.

To reduce the emergence of the antibiotic resistance microbes, use of antimicrobials should be monitored by infection control unit in hospitals (2). The rapid increase in multidrug resistant MDR bacterial strains was observed, which may be due to the poor applied programs (3). Patients excessive use of antibiotics, whether on their own or within the hospital is a major factor for the emergence of resistant strains (4). The increasing use of antimicrobials in humans, animals, and agriculture has resulted in developing many pathogens resistant to these powerful drugs (5).

Resistant bacteria are divided according to the group of antibiotics, they are resistant to. conjugation of a plasmid is the most prevalent type for the acquisition of resistance among bacteria (6). Recently, the latest resistance mechanisms have resulted in the emergence of MDR bacterial strains (7). From now on, new antimicrobial agents are needed to eliminate the infection resulted from multidrug resistant microbes and potential use against humans during wars (8). Controlling the spread of resistance requires the collaboration of several organizations such as veterinary, medical, and public health communities (9). If the microbe is resistant to more than a class of antibiotics, it is considered a multidrug resistance, and the choice of drug against microbe is mainly associated with the knowledge of its sensitivity, to gain better results in the treatment of

patients (10).

2. Objectives

The aim of this study was to isolate, identify, and characterize the prevalence of clinical isolates along with their antimicrobial sensitivity pattern among the patients referring to King Khalid hospital, Saudi Arabia.

3. Patients and Methods

3.1. Study Area

A total of 428 clinical samples such as pus-swap, urine, blood, suction tip, catheter tip, pleural fluid, bronchial wash, peritoneal fluid, ear swap, and sputum were collected from the patients of King Khalid hospital as a part of routine patient care. The surveillance methodology was carried out according to Emori et al. study (11). In brief, the sample collection started in June 2013 and continued for 8 months. They were collected from 8 medical and surgical wards, including intensive care unit, female medical ward, male medical ward, male surgical ward, maternity and child care, obstetrics ward, pediatric ward, artificial kidney unit, and nursery. Then, the samples were immediately transferred to the laboratory for further processing.

3.2. Samples Processing and Antibiogram

Samples were plated on blood agar (BA), MacConkey agar (MAC), cetrimide agar (CA), and nutrient agar (NA) (bought from Aldrich Chemical, Milwaukee, WI, USA). Then, they were incubated at 37°C for 48 hours. A total of 300 cultures were subjected to MicroScan for identification and antibiotic susceptibility. We used MicroScan instrumentation (auto SCAN-4 and WalkAway System) (Siemens healthcare diagnostics Inc, USA). Panels used in the study were MicroScan dried Gram-positive MIC/Combo, dried Gram-positive breakpoint combo, and dried Gram-positive ID Type 2 or 3. Also, MicroScan dried Gram-negative MIC/Combo panels and dried Gram-negative breakpoint Combo panels were used. These panels were designed for use in determining agent susceptibility and or identification of the species, level of rapidly growing aerobic and facultative Gram-positive cocci, or aerobic and facultative anaerobic Gram-negative bacilli. The tests were performed as recommended by supplier guidelines (12). Susceptible, intermediate, and resistant isolates were arranged by location in the hospital according to antibiogram results.

3.3. Bacterial Isolates and Confirmation

A total of 300 non-duplicate clinical isolates of selected organisms were collected and each one was accompanied by antibiogram indicating susceptibility and resistance pattern. Bacterial isolates were subjected to re-identification using morphological and biochemical tests. Also, validation test was conducted on 283 isolates, whereas 17 isolates were lost or not purified. The results of this validation test provided a sense of the accuracy of the laboratory method of the susceptibility test in identifying organisms as resistant. In validation test, MICs were employed by the standards of antimicrobial susceptibility testing according to CLSI guidelines (13). MIC-derived by MicroScan was compared to the results of validation test. Organisms defined as causing major errors were those categorized by MicroScan as resistant and found to be susceptible by validity testing. Organisms defined as causing minor errors were classified as intermediate by validity test and as susceptible or resistant by MicroScan or classified as intermediate by MicroScan and as susceptible or resistant by validity testing. Antibiotic susceptibility test was done on these isolates to determine the susceptibility of the isolate to an array of antibiotics, which would determine the extent of resistance or sensitivity of the organism to each antibiotic.

4. Results

The study was carried out at the laboratory in the college of applied medical sciences and King Khalid hospital over a period of 8 months from June 2013 to January 2014. During the entire study period, a total of 300 clinical isolates along with their antibiogram reports were collected.

4.1. Infection Evaluation

Of 428 clinical samples, 300 bacterial isolates were obtained, which accounting for an isolation rate of 70.1%. A total of 300 infectious isolates were yielded as monobacterial growth. Of them, 116 (39.0%) isolates were isolated and identified from wounds, 97 (32.3%) from urine, 54 (17.8%) from blood, 20 (6.7%) from suction tip, 6 (2.0 %) from catheter tip, 2 (0.8%) from purulent discharge, and 1 (0.3%) from each bronchial wash, peritoneal fluid, ear swab, pleural fluid, and sputum (Table 1).

The culture reports revealed that Gram-negative organisms like *K. pneumonia*, *E. coli*, and *P. aeruginosa* were the predominant organisms, (77.3%, n = 232), followed by Gram-positive organisms like *Staphylococcus aureus* (22.7%, n = 68) (Table 1). The culture reports also specified the microorganisms isolated from each specimen. Data indicated the highest prevalence of *E. coli* and *S. aureus* over all other organisms during the study period. Isolates of *E. coli* were found

Table 1. Common Pathogens Associated With Specimens Collected From Patients in King Khaled Hospital in Hafr Al-Batin

Bacterial Isolates	Specimen Sources											Total, No. (%)
	Wound Swab	Urine	Blood Culture	Suction Tip	Catheter Tip	Purulent Discharge	Bronchial Wash	Peritoneal Fluid	Ear Swab	Pleural Fluid	Sputum	
Gram-positive												
S. aureus	36	ND	20	12	ND	ND	ND	ND	ND	ND	ND	68 (22.7)
Gram-negative												
E. coli	39	61	7	4	1	1	ND	1	ND	1	ND	115 (38.3)
K. pneumonia	17	18	20	4	3	1	ND	ND	1	ND	1	65 (21.7)
P. aeruginosa	24	18	7	ND	2	ND	1	ND	ND	ND	ND	52 (17.3)
Total, No. (%)	116 (39)	97 (32.3)	54 (17.8)	20 (6.7)	6 (2)	2 (0.8)	1 (0.3)	1 (0.3)	1 (0.3)	1 (0.3)	1 (0.3)	300 (100)

to be 38.3% (n = 115) of all obtained isolates while *S. aureus* was found to comprise 22.7% (n = 68) isolates. On the other hand, *K. pneumonia* and *P. aeruginosa* rates were 21.7% (n = 65) and 17.3% (n = 52), respectively (Table 1). Also, majority were isolated from pus/swap, urine, and blood samples. A total of 283 non-duplicate clinical isolates containing 4 various microorganisms were underwent susceptibility testing against various antibiotics.

4.2. Antibiotic Susceptibility Testing

Resistant and susceptibility profile of *S. aureus* showed its high resistance to both ampicillin and linezolid (94.1%) and high sensitivity to more than one antibiotic such as daptomycin, penicillin, Synercid, teicoplanin, vancomycin, and Trimethoprim-sulfamethoxazole, which have sensitivity rate more than 88% (Table 2).

Klebsiella pneumonia was fully resistant to ampicillin (100%) followed by mezlocillin and piperacillin (92.3%) and showed highest sensitivity to imipenem (84.61%) followed by amikacin and piperacillin-tazobactam (76.92%). *Pseudomonas aeruginosa* was fully resistant to 4 antibiotics of cefazoline, cefoxitin, tetracycline and trimethoprim/sulfamethoxazole (100%) (Table 3). This indicates high incidence of MDR of *P. aeruginosa*. On the other hand, *E. coli* isolates were highly resistant (more than 78%) to several antibiotics such as amikacin, ampicillin, mezlocillin, and piperacillin. Also, *E. coli* isolates had the highest sensitivity (more than 86%) to fosfomycin, imipenem, piperacillin/tazobactam, and tigecycline (Table 3).

4.3. Validation of Resistant Isolates

Isolates subjected to validation test are shown in Table 4. The frequencies of minor or major errors were within an acceptable range. Validity test by broth microdilution of 107 amikacin-, ampicillin-, mezlocillin-, or piperacillin-resistant *E.coli* sent by the hospital revealed 2 minor errors (1.8%) and 2 major errors (1.8%). Also, testing 60 ampicillin-, mezlocillin-, or piperacillin-resistant *K. pneumonia* isolates showed 4 minor errors (6.7%). On the other hand, for validity testing of 64 methicillin-resistant *S. aureus* (MRSA) or 52 cefazoline-, cefoxitin-, tetracycline-, or trimethoprim-sulfamethoxazole-resistant *P. aeruginosa* isolates, no errors were detected. The rate of errors varied slightly between the two test methods; MicroScan and broth microdilution susceptibility testing. Results also showed increasing number of multiple-drug resistant (MDR) isolates in Female surgical, 100%; ICU 90.2%; Male surgical, 88.2%; Obstetrics and Nursery, 75.0%; Pediatrics 69.2%; and Artificial kidney wards, 54.2% (Table 4).

5. Discussion

Contraction of the infection during the health care, often leads to poor prognosis, increase mortality, and health care costs. Therefore, execution of an integrated program to reduce the infection (30%) will decline the health care costs (14).

Studies carried out by different researchers have reported varied isolation rates. In the present study, high isolation rate (70.1%) was obtained from clinical samples. Sidhu et al. (15) reported an isolation rate of 45.9%, while Vijaya et al. (16) reported it to be 21.8%. In a study from Saudi Arabia, Eltahawy and Khalaf (17) reported 16% of all the Gram-negative bacilli isolated. The present study included the types and antibiotic susceptibility pattern of bacterial organisms isolated from different samples of patients in King Khalid hospital.

Table 2. Antimicrobial Susceptibility Pattern of Gram Positive Bacteria

Antibiotic	Gram-Positive Bacteria Isolates (*S. aureus*) (n = 68)		
	Resistant	Intermediate	Sensitive
Amoxicillin/clavulanic	38 (55.9)	0	30 (44.1)
Ampicillin	64 (94.1)	4 (5.9)	0
Azithromycin	34 (50.0)	4 (5.9)	30 (44.1)
Ciprofloxacin	30 (44.1)	0	38 (55.9)
Clindamycin	20 (29.4)	0	48 (70.6)
Daptomycin	0	7 (10.3)	61 (89.7)
Erythromycin	30 (44.1)	8 (11.8)	30 (44.1)
Fosfomycin	13 (19.1)	0	55 (80.9)
Fusidic Acid	4 (5.9)	26 (38.2)	38 (55.9)
Gentamicin	12 (17.6)	0	56 (82.4)
Imipenem	43 (63.2)	0	25 (36.8)
Levofloxacin	17 (25.0)	0	51 (75.0)
Linezolid	64 (94.1)	4 (5.9)	0
Moxifloxacin	18(26.5)	4 (5.9)	46 (67.6)
Mupirocin	13 (19.1)	0	55 (80.9)
Oxacillin	38 (55.9)	0	30 (44.1)
Penicillin	4 (5.9)	0	64 (94.1)
Rifampin	8 (11.8)	0	60 (88.2)
Synercid	4 (5.9)	0	64 (94.1)
Teicoplanin	4 (5.9)	0	64 (94.1)
Tetracycline	25 (36.8)	0	43 (63.2)
Trimethoprim/sulfa	8 (11.8)	0	60 (88.2)
Vancomycin	4 (5.9)	0	64 (94.1)

The bacteriological methods for isolation and identification resulted in 300 isolates, 116 from wounds, 97 from urine, 54 from blood, 20 from suction tip, 6 from catheter tip, 2 from purulent discharge, and 1 from each of bronchial wash, peritoneal fluid, ear swab, pleural fluid, and sputum. Of these isolates, *E. coli* was the most common with 115 (38.3%) isolates (61 from urine, 39 from wound, 7 from blood, 4 from suction tip, and 1 from each catheter, purulent discharge, peritonea fluid and pleural fluid) followed by *S. aureus* with 68 (22.7%) isolates (36 isolated from wound, 20 from blood and 12 from suction tip). On the other hand, isolates of *K. pneumonia* and *P. aeruginosa* were 65 (21.7%) and 52 (17.3%), respectively. Similar results were obtained by Samonis et al. (18). They found that E. coli was the most common organism isolated from pus (47.05%) and its resistant rate was 50.0% followed by *S. aureus* (29.41%) with the resistant rate of 60.0%. Similar observations were previously recorded (19).

Antibiotic susceptibility profile of *S. aureus* showed its high resistant to ampicillin and linezolid. Also, it has high sensitivity to more than one antibiotic, including penicillin, Synercid, tetracycline, trimethoprim/sulfamethoxazole, and vancomycin. These results were similar to those obtained by Tesfaye et al. (20) and Bharathi et al. (21). *K.pneumonia* isolates were fully resistant to ampicillin (100%) and very sensitive to imipenem (84.61%). Similar results were obtained by Okonko et al. (22).

Pseudomonas aeruginosa was fully resistant to 4 antibiotics: cefazoline, cefoxitin, tetracycline, and trimethoprim/sulfamethoxazole. This result indicates high incidence of MDR-isolated clinical bacteria. Similar high rate (84%) was reported by Dash et al. (23). Likewise, the findings of Okonko et al. (22) who reported MDR to 5 antibiotics (ampicillin, chloramphenicol, Co-trimoxazole, nitrofurantoin, and tetracycline). Multi-resistance *P. aeruginosa* was also isolated by Olowu and Oyetunji (24) and Fagade et al. (25). Also, Aiyegoro et al. (26), isolated multi-resistance *P. aeruginosa* in their study to determine the incidence of urinary tract infection in children and adolescents. The higher percentage of MDR isolates from different clinical specimens will become problematic in the future.

On the other hand, Synercid, teicoplanin, and vancomycin were the most effective drugs against *S. aureus* observed in this study. It showed resistance rate of 5.9%; low resistance rate of *S. aureus* may be due to the recent intro-

Table 3. Antimicrobial Susceptibility Pattern of Gram-Negative Bacteria[a]

Antibiotic	Gram-Negative Bacterial Isolates (n = 232)								
	E. coli (n = 115)			*K. pneumonia* (n = 65)			*P. aeruginosa* (n = 52)		
	Resistant	Intermediate	Sensitive	Resistant	Intermediate	Sensitive	Resistant	Intermediate	Sensitive
Amikacin	90 (78.26)	0	25 (21.73)	15 (23.07)	0	50 (76.92)	26 (50)	0	26 (50)
Amox-Clav	35 (30.43)	20 (17.39)	60 (52.17)	35 (53.84)	10 (15.38)	20 (30.76)	32 (61.5)	0	20 (24.4)
Ampicillin	100 (86.95)	0	15 (13.04)	65 (100)	0	0	32 (61.5)	0	20 (24.4)
Cefazoline	55 (47.82)	0	60 (52.17)	50 (76.92)	0	15 (23.07)	52 (100)	0	0
Cefepime	50 (43.47)	0	65 (56.52)	50 (76.92)	0	15 (23.07)	32 (61.5)	0	20 (24.4)
Cefotaxime	50 (43.47)	0	65 (56.52)	NT	NT	NT	45 (86.5)	0	7 (13.5)
Cefoxitin	40 (34.78)	0	75 (65.21)	30 (46.15)	0	35 (53.84)	52 (100)	0	0
Cefuroxime	50 (43.47)	5 (4.34)	60 (52.17)	50 (76.92)	0	15 (23.07)	45 (86.5)	0	7 (13.5)
Ciprofloxacin	70 (60.86)	5 (4.34)	40 (34.78)	40 (61.53)	5 (7.69)	20 (30.76)	39 (75)	0	13 (25)
Ertapenem	20 (17.39)	0	95 (82.6)	15 (23.07)	15 (23.07)	35 (53.84)	34 (65.5)	12 (25)	6 (12.5)
Fosfomycin	10 (8.69)	0	105 (91.3)	25 (38.46)	0	40 (61.53)	32 (61.5)	0	20 (37.5)
Gentamicin	55 (47.82)	5 (4.34)	55 (47.82)	30 (46.15)	0	35 (53.84)	38 (75)	0	14 (27)
Imipenem	5 (4.34)	5 (4.34)	105 (91.3)	10 (15.38)	0	55 (84.61)	19 (36.5)	7 (13.5)	26 (50)
Levofloxacin	60 (52.17)	0	55 (47.82)	25 (38.46)	5 (7.69)	35 (53.84)	39 (75)	0	13 (25)
Mezlocillin	90 (78.26)	5 (4.34)	20 (17.39)	60 (92.3)	0	5 (7.69)	39 (75)	0	13 (25)
Pip-Tazo	15 (13.04)	0	100 (86.95)	15 (23.07)	0	50 (76.92)	20 (38.5)	0	32 (61.5)
Piperacillin	90 (78.26)	0	25 (21.73)	60 (92.3)	0	5 (7.69)	NT	NT	NT
Tetracycline	75 (65.21)	0	40 (34.78)	30 (46.15)	5 (7.69)	30 (46.15)	52 (100)	0	0
Tigecycline	15 (13.04)	0	100 (86.95)	15 (23.07)	5 (7.69)	45 (69.23)	NT	NT	NT
Tobramycin	65 (56.52)	5 (4.34)	45 (39.13)	40 (61.53)	0	25 (38.46)	34 (65.4)	6 (11.5)	12 (23.1)
Trimeth/Sulfa	75 (65.21)	0	40 (34.78)	40 (61.53)	0	25 (38.46)	52 (100)	0	0

Abbreviations: Amox-Clav, Amoxicillin-clavulanic; Pip-Tazo, Piperacillin-Tazobactam; Trimeth-Sulfa, Trimethoprim-Sulfamethoxazole; NT, not tested.
[a] Values are expressed as No. (%).

Table 4. Distribution of MDR Clinical Bacterial Isolates by Ward in King Khalid Hospital[a,b]

Antibiotic-Resistant Organism	Ward							
	ICU	Female Surgical Ward	Male Surgical Ward	Obstetrics Ward	Pediatric Ward	Artificial kidney Unit	Nursery	Total
Methicillin-resistant *S. aureus*	8/8 (100)	32/32 (100)	16/16 (100)	ND	ND	8/8 (100)	ND	64/64 (100)
Ampicillin-resistant *K. pneumonia*	16/16 (100)	8/8 (100)	19/20 (95.0)	4/4 (100)	3/4 (75.0)	ND	6/8 (75.0)	56/60 (93.3)
Amikacin-, Ampicillin-, Mezlocillin-, or Piperacillin-resistant *E. coli*	11/17 (64.7)	28/28 (100)	20/29 (68.9)	11/16 (68.7)	6/9 (66.7)	5/8 (62.5)	ND	81/107 (75.70)
Cefazoline-, Cefoxitin-, Tetracycline-, or Trimeth/Sulfa-resistant *P. aeruginosa*	20/20 (100)	4/4 (100)	20/20 (100)	ND	ND	8/8 (100)	ND	52/52 (100)
Total	55/61 (90.2)	72/72 (100)	75/85 (88.2)	15/20 (75.0)	9/13 (69.2)	13/24 (54.2%)	6/8 (75.0)	253/283 (89.4)

Abbreviations: ICU, intensive care unit; ND, the microbe not detected in that area; Trimeth-Sulfa, Trimethoprim-Sulfamethoxazole.
[a] Data are presented as No. of resistant isolates.
[b] Total No. of isolates tested (%).

duction of this antibiotic. Similar results were obtained by Japoni et al. (27) for vancomycin. Our study showed no effective drugs against *K. pneumonia* and *P. aeruginosa*, whereas the lowest resistance rates were 15.38% and 36.5%

for imipenem. Accurate laboratory detection and control of patient to patient transmission are cornerstones in containment of drug resistant. The higher rates of resistance in ICU and surgical wards may be parallel with higher usage of antimicrobial drugs. Other factors such as use of other drugs or cross-transmission may play an important role in propagation of these organisms.

Our study showed high drug resistant rate. Drug resistant may be due to infection control practices, inadequate antibiotic treatment, or noncompletion of treatment course that may lead to infection recurrent and drug resistance. Drug susceptibility varied between the hospital wards. We believe that reporting antimicrobial use must be stratified by hospital wards to make valid comparisons between areas. Further studies are required to determine the importance of specific ICU type as well as regional variations in the patterns of antimicrobial use. Although this surveillance assessed antimicrobial agents in a number of specimens, more research is needed to clarify the reasons of drug resistance and its prevalence in Saudi Arabia hospitals.

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