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Biochemical and molecular characterizations of multidrug resistance bacteria isolated from Saudi Arabian hospital

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One of the well-recognized problems nowadays is the epidemic of the resistance of microorganisms to antimicrobial agents. So, this descriptive, cross-sectional, hospital-based study starts to screen the patient's samples to search for most antibiotic-resistant bacteria. A total of 120 isolates from outpatients and inpatients were used to identify the most MDR bacteria. A 120 samples was collected at King Abdullah Medical Complex. VITEK® 2 (BioMérieux, France) and MicroScan Microbiology system (Siemens, Germany) were used in isolates identification and antimicrobial susceptibility test. So, the highest prevalence of bacterial isolates was identified as *Klebsiella pneumoniae* 26 (21.66%), *Escherichia coli* 23 (19.16%), *Pseudomonas aeruginosa* 16 (13.33%) and *Staphylococcus aureus* 15 (12.5%). The most resistant sample was selected based on the number of antibiotics it was susceptible to. *K. pneumoniae*, found to be resistant to 16 antibiotics. Furthermore, the isolate is identified as OXA-48-Positive Carbapenem-Resistant *K. pneumoniae*.

Keywords: MAR, Carbapenem-Resistant Klebsiella pneumoniae, Antibiotic resistance, OXA-48 gene

INTRODUCTION

Past introduction here.

In modern medicine, antimicrobial agents are recognized to be the best therapy to treat infectious diseases. In the time of pre-antibiotics, the percentage of surviving any bacterial infections is remarkably low; also, amputation is common practice in the case of infected wounds (Friedman et al. 2015). However, multiple drug resistance bacteria are becoming an obstacle that have to face in the coming years. This complication will disturb the health of both humans and animals. Additionally, caesarean section, chemotherapy and other vital surgical interventions will be affected and might be limited due to these undesirable occurrences of bacterial resistance to antibiotics (Zowawi, 2016). Over forty years ago, *Shigella spp. and Escherichia coli* multiple drug resistance markers were initially described. Besides, between strains of bacteria and other species plasmids that carry those MDR genes known to be self-transfer by conjugation, therefor it makes multi-resistance bacteria significant threat to the globe (Hawkey and Jones, 2009).

On the other hand, mortality and morbidity in patients are linked to multiple drug resistance hospital-acquired infections or as known

nosocomial infections. Moreover, intensive care unit patients are more critical to acquire infection due to poor health and extensive use of antibiotics (Mehta et al. 2015).

In addition to that, Surgical site infections are emerging, and one of the common causing pathogens are gram-negative pathogens (Olowo-okerea et al. 2018).

In consequence, the evolution of bacteria and its ability to be resistant to antimicrobial agents raised the need to pursue an alternative therapy or discover new antibiotics (Stanton, 2013). However, the pharmaceutical industry, academia and health institutions requisite to lookout any immediate solutions through research in hope for a new discovery to diminish such issue (Cavera et al. 2015). Therefore, the other aim of this research was to isolate and identify the most resistant bacteria then perform molecular characterization of the resistance genes of the isolate in Jeddah, Saudi Arabia.

MATERIALS AND METHODS

The antibiotics were used demonstrated in table 1.

2.1 Samples collection and Preparation

Fresh samples of biological specimens from intensive care unit (ICU) and hospital clinics (includes Blood, Urine, and Sputum) have been collected from King Abdullah Medical Complex (K.A.M.C) in Jeddah, Saudi Arabia. The urine and sputum samples moved to the streaking area (Class II type B2 biological safety cabinets) to be processed. However, the blood sample scanned in the BACT/ALERT® 3D machine (BioMérieux, France), then it was loaded and incubated for 1 to 5 days.

2.2 Samples processing

2.2.1. Urine Samples

The streaking area is prepared with the proper medium plates (Blood sheep W/ CLED agar), then the urine containers brought to the safety cabinet with the labeled barcode that holds the patient's information. A disposable loop dipped into the urine sample, start streaking into the sheep blood agar afterward the CLED by drawing a line from top to bottom, then the plates will be incubated at 37 ^{o}C for 24-48 hours.

2.2.2. Blood Samples

For the blood samples process starts when the BACT/ALERT® 3D machine (BioMérieux, France), release an alert of a positive sample.

Table 1. List of antibiotics used in the study.

No.	Antibiotic	Abb.
1	Amikacin	AMK
2	Amoxicillin-clavulanic acid	AMC
3	Ampicillin	AMP
4	Ampicillin-sulbactam	SAM
5	Benzylpenicillin	PEN G
6	Cefoxitin	FOX
7	Ceftazidime	CAZ
8	Ceftriaxone	CRO
9	Cefuroxime	CXM
10	Cefalotin	INN
11	Levofloxacin	LVX
12	Linezolid	LZD
13	Meropenem	MEM
14	Moxifloxacin	MXF
15	Nitrofurantoin	NIT
16	Piperacillin-tazobactam	TZP
17	Quinupristin-dalfopristin	QD
18	Streptomycin	STR
19	Teicoplanin	TEC
20	Tetracycline	TET
21	Ciprofloxacin	CIP
22	Clindamycin	CLI
23	Erythromycin	ERY
24	Gentamicin	GEN
25	Imipenem	IPM
26	Cefepime	CPE
27	Vancomycin	VAN
28	Oxacillin	OXA
29	Colistin	CLN
30	Tigecycline	TGC

The sample will be removed from the machine to the safety cabinet to be cultured. Using three mediums (Blood medium, Chocolate medium and MacConkey medium) plus a slide. After labeling all plates with patent's info, a deflated syringe was used once the blood bottle swabbed with an alcohol swab to draw a 1 to 3 ml of the blood. Next, two drops of the sample placed into the medium's plates and the slide. A disposable loop was used for streaking. Following, the plates were

incubated for 24-48 hrs., at 37 °C to perform the gram staining process.

2.2.3. Sputum Samples

The mediums (Blood medium, Chocolate medium and MacConkey medium) were used, also a disposable loop, cotton swab and the labeled sample. Beginning with a cotton swab that dipped into the sample container and mix to be sure the sputum is stick to it. Then by a circular motion, the sample was smeared into the three mediums, each time dip the swab to get more sputum. Then discard the swab and take a loop and start streaking, the plates are incubated for 24-78 hrs., at 37 °C.

2.3. Identification of isolates

The previous urine and sputum cultured samples are reviewed and read after the incubation period, to determine to wither the bacteria is gram positive or negative. First, in sputum samples reading the morphology and smell of colonies, also using selective media such as MacConkey medium for gram negative, which is mainly used for *Enterobacteriaceae* and *Pseudomonas spp.*. However, the urine samples identification wither isolates are gram negative or positive is revealed by the morphology of the colonies, coagulase test, oxidase test and the CLED medium. Then, automated Identification was made by VITEK® 2 COMPACT (BioMérieux, France) and MicroScan Microbiology system (Siemens, Germany).

2.4. Susceptibility Testing

The Antimicrobial susceptibility testing (AST) of the isolates was done on a fully Automated process completed by VITEK® 2 COMPACT (BioMérieux, France) and MicroScan system (Siemens, Germany). Besides, some samples were processed manually using the antibiotic susceptibility discs of Mastring-S™ (MAST Group Ltd.UK). After the automation process identifies the culture, a subculture is made and streaked with a swab on all over the Mueller-Hinton Agar plate, later a proper disc of (GN or GP) Antibiotics were placed in the plate, then incubated for 24-48 hrs., at 37 °C.

2.5. Molecular Characterization

The selected molecular assay is Xpert Carba-R Assay, performed on the GeneXpert® Instrument Systems, (Cepheid Inc, USA), a qualitative *in vitro* diagnostic test designed for the detection and differentiation of the *blaKPC, blaNDM, blaVIM, blaOXA-48 and blaIMP* gene sequences associated with carbapenem-non-susceptibility. The test utilizes automated real-time polymerase chain reaction (PCR). Thus, the sample prepared regarding the system's protocol.

2.6. Statistical Analysis

Pearson Chi-Square test was performed using IBM SPSS® (IBM-SPSS Inc, Armonk, NY)., 20 software for frequency distribution significance with 95% confidence interval.

RESULTS

3.1. Isolation and Identification of the Most Multiple-Drug Resistance Bacteria

A total of 120 samples were collected and screened for the most multiple-resistance bacteria. The samples were grouped as the following; inpatients (urine 30, blood 30 and sputum 30), outpatients (urine 30). The percentage of the inpatients' samples was 75%; the outpatients' samples was 25%. In addition, the age group of the samples to gender is demonstrated in table 2. As was shown, the youngest participant was an outpatient, 19-year-old who is female, whereby the eldest was a hospitalized 98-years-old male. Also, the resistance percentage is calculated and appeared to be high; however, it was highest in the age group of participants who older than 90 with 100% bacterial resistance to multiple antibiotics. Moreover, the percentage of the participant's gender is shown in table 3.

First, in inpatients samples, numerous bacterial species were found in three specimens' categories, such as *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Proteus mirabilis, Enterococcus faecalis, Stenotrophomonas maltophilia, Escherichia coli and Streptococcus agalactiae*. Most of the cultures presented a growth of colonies within 24-48hrs. Accordingly, most bacterial isolates were *K. pneumoniae* with 26 isolates, and the least isolates were to be equal to 1 in several bacterial such as *Acinetobacter Iwoffii, Serratia marcescens, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis* and *Stenotrophomonas maltophilia*, as shown in figure 1. Table 3 displays the distribution of the bacterial isolates according to the gram staining. A 67.5% gram- negative bacteria and 32.5% gram positive. The most MDR revealed after the full collection of 120 samples and the comparison of the antibiotic susceptibility patterns.

Table 2. Distribution of age group to gender and the susceptibility percentage.

	Gender		Sus.			
Age Group	**Male**	**Female**	**MDR**	**Not MDR**	**Total**	**Percentage of Resistance**
19-29	3	6	6	3	9	66.60%
30-39	9	4	12	1	13	92.30%
40-49	7	5	11	1	12	91.60%
50-59	3	11	12	2	14	85.70%
60-69	7	13	19	1	20	95%
70-79	11	16	23	4	27	85.10%
80-89	5	16	20	1	21	95.20%
90+	3	1	4	0	4	100%
Total	48	72	107	13	120	

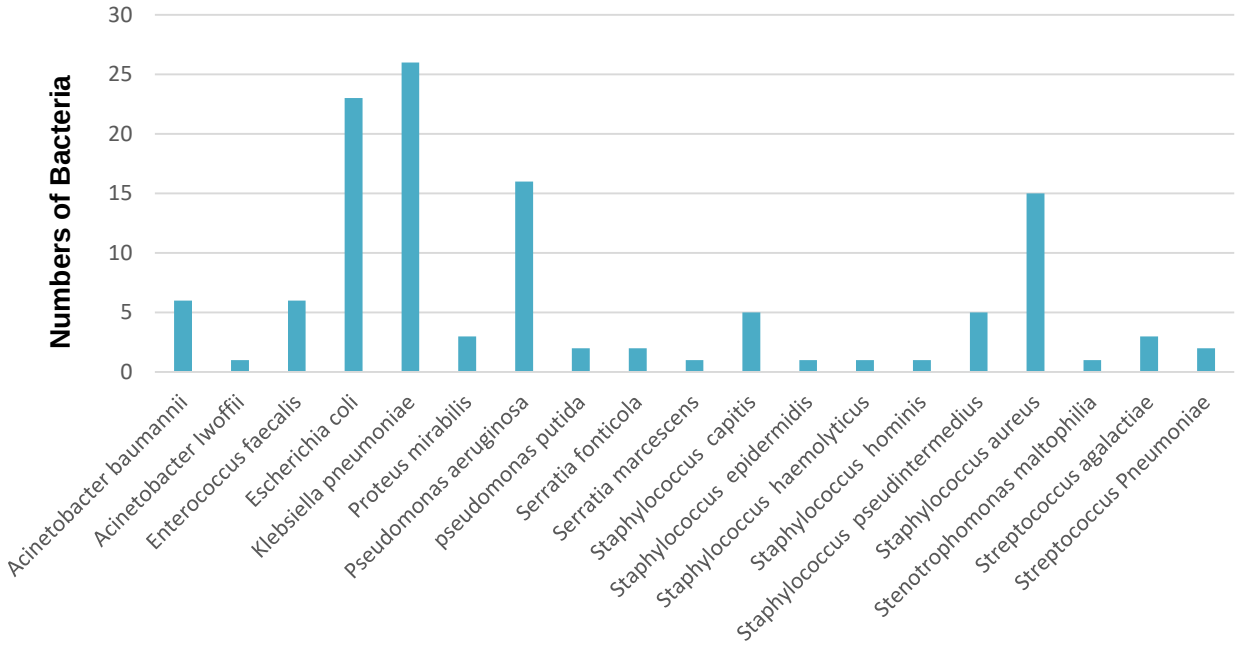

Figure 1. A Bar Charts Displays the Numbers of Bacterial Isolates.

A 95 years old, male who was an ICU patient, found to have a *K. pneumoniae* that presented property of carbapenem-resistant Enterobacteriaceae (CRE). The bacteria were found in his urine culture, its chrematistics gram-negativee rods, non-motile, non-spore forming, catalase-positive, oxidase negative and facultatively anaerobic. Morphology described as shown in figure 2; colonies were appeared in large, single and also in pairs, short chains, yellow or yellowish-white on CLED agar medium and non- hemolytic grey-white, mucoid colonies on sheep blood agar medium. The pathogenicity of *K. pneumoniae* known to be opportunistic pathogens and it spreads mainly in hospitals.

Figure 2. *K. pneumoniae* growing on Sheepblood (a), and CLED agar media(b).

Table 3. Gram Reaction Percentage of Bacterial Isolates.

Gram Type	Number	Percentage
Gram Negative	82	67.5%
Gram Positive	38	32.5%
Total	n= 120	100%

3.2. Susceptibility Test for the Most Multiple-Drug Resistance Bacteria

The results obtained in figure 3 represent that 31 antibiotics were used in susceptibility test; for these antibiotics 9% ampicillin, 7% CPE, CAZ and LVX. linezolid presented a high sensitivity with zero percent resistance. Approximate of 107(74%) isolates were found to be MDR and 13(26%) were not MDR, figure 4

The categories (inpatients and outpatients) susceptibly patterns were investigated. Table 5 demonstrated the inpatients' blood and sputum samples, and it reported the following; out of five *S. aureus* isolates from blood, only one was found to be methicillin-resistant *Staphylococcus aureus* MRSA. Its resistance pattern was as following; levofloxacin, amoxicillin-clavulanic acid, ampicillin, ciprofloxacin, moxifloxacin and oxacillin, and two out of the seven *S. aureus* isolates were MRSA it belonged to a 74- and 80-years old males with shared patterns of resistance in oxacillin and benzylpenicillin, and sensitivity to vancomycin. As it is presented in table 4, the blood samples *K. pneumoniae* four isolates shared a common sensitivity for amikacin, imipenem and meropenem. However, in sputum *K. pneumoniae* patterns are presented in 6 different isolates, one of which belonged to 79 years- old male that revealed resistance to 13 various antibiotics. Also, two females participants, 83- and 58 years-old, shared the same resistance patterns with ampicillin resistance only.

The susceptibly patterns of urine samples (inpatients and outpatients) were also studied. The inpatients presented significant resistance patterns in comparison with the outpatients. As in table 4, a 95 years-old with *K. pneumoniae*, which considered a carbapenem-resistant Enterobacteriaceae CRE, shown the most resistance patterns out of all the 120 isolates with 16 antibiotics resistance and no sensitivity at all. On the other hand, nine patterns of *K. pneumoniae* in the outpatients' group showed a common resistance to ampicillin only, and common sensitivity to imipenem, meropenem, cefoxitin and tigecycline. Besides, table 5 showed that *S. aureus* isolated from 49 years-old male presented resistance to oxacillin and benzylpenicillin and more, it considered to be a MRSA, and it was sensitive to vancomycin as well as tetracycline, clindamycin, linezolid teicoplanin and nitrofurantoin. Conversely, A 69-year-old female who was admitted from the emergency room exhibited a methicillin-resistant *Staphylococcus aureus* MRSA, and its sensitivity patterns ensured a sensitivity towards vancomycin, nitrofurantoin and trimethoprim-sulfamethoxazole.

The results indicated an observable difference between hospitalization status and

MDR, where in-patient subjects exhibited a much higher rate of MDR than out-patient (p = 0.15).

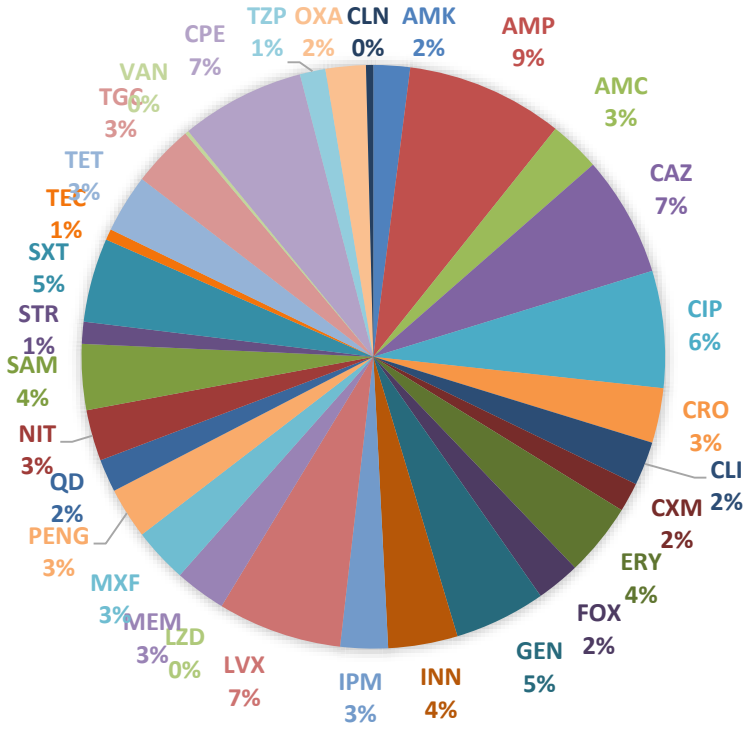

Figure 3. Total Resistance Percentage of Bacterial Isolates to Testes Antibiotics

See figure 5, a representative of this set of data as pie-of-pie chart, where the two sets of data for In-P and Out-P is displayed in detail.

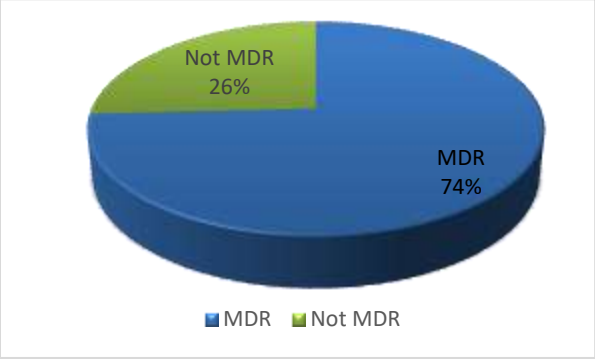

Figure 4. Percentage of Multiple-drug Resistance in Isolated Bacteria

3.4. Molecular Characterization

The results are interpreted by the GeneXpert System from measured fluorescent signals and embedded calculation algorithms. The interpretations of the achieved results as following; OXA-48 target DNA sequence is detected and IMP, VIM, NDM and KPC target DNA sequences are not detected. PCR amplification of the OXA-48 target DNAs give Ct value (19.2) within the valid ranges and fluorescence endpoints (355) above the threshold settings; IMP, VIM, KPC and NDM target DNA sequences are absent or below the assay detection level as in table 6.

DISCUSSION

Since the power of Antimicrobial agents is threatened to be ineffective therapy for microbial infections due to the increase of resistance genes spreading through mobile genetic elements like plasmids and transposons (Leski et al. 2013). Since the extreme mishandling of antibiotics in the past and currently in some countries around the globe, this problem becomes even tougher to tackle (Al-Shibani et al. 2017). This study concentrated on screening the patterns of susceptibility then characterizing the finding biochemically and molecularly. Overall, the data suggest that multiple drug resistance concern is present and intimidating. For instance, in a total of 120 isolates, a 107 bacterial isolate found to be multiple drug resistance and only 13 is not.

Table 4. Antibiotic susceptibility pattern of *K. pneumoniae*.

Antibiotics	K. pneumoniae from Blood n=4			K. pneumoniae from Sputum n=6			K. pneumoniae from In.P Urine n=7			K. pneumoniae from Out.P Urine n=9		
	Sensitive no. (%)	Intermediate no. (%)	Resistant no. (%)	Sensitive no. (%)	Intermediate no. (%)	Resistant no. (%)	Sensitive no. (%)	Intermediate no. (%)	Resistant no. (%)	Sensitive no. (%)	Intermediate no. (%)	Resistant no. (%)
AMC	1 (25%)	1 (25%)	1 (25%)	2 (33.3%)	0 (00.0%)	1 (16.6%)	5 (71.4%)	0 (00.0%)	2 (28.57%)	5 (55.55%)	2 (22.2%)	1 (11.1%)
AMK	4 (100%)	0 (00.0%)	0 (00.0%)	5 (83.3%)	0 (00.0%)	1 (16.6%)	2 (28.57%)	0 (00.0%)	5 (71.4%)	6 (66.66%)	1 (11.1%)	2 (22.2%)
AMP	0 (00.0%)	0 (00.0%)	3 (75%)	0 (00.0%)	0 (00.0%)	5 (83.3%)	0 (00.0%)	0 (00.0%)	7 (100%)	0 (00.0%)	0 (00.0%)	9 (100%)
CAZ	1 (25%)	0 (00.0%)	3 (75%)	3 (50%)	0 (00.0%)	3 (50%)	3 (42.85%)	0 (00.0%)	4 (57.14%)	5 (55.55%)	0 (00.0%)	3 (33.3%)
CIP	2 (50%)	0 (00.0%)	2 (50%)	4 (66.6%)	0 (00.0%)	2 (33.3%)	5 (71.4%)	0 (00.0%)	2 (28.57%)	3 (33.3%)	0 (00.0%)	2 (22.2%)
CRO	1 (25%)	0 (00.0%)	1 (25%)	1 (16.6%)	0 (00.0%)	0 (00.0%)	2 (28.57%)	0 (00.0%)	2 (28.57%)	1 (11.1%)	0 (00.0%)	3 (33.3%)
CXM	0 (00.0%)	0 (00.0%)	1 (25%)	2 (33.3%)	0 (00.0%)	1 (16.6%)	1 (14.28%)	1 (14.28%)	1 (14.28%)	4 (44.4%)	0 (00.0%)	3 (33.3%)
FOX	1 (25%)	0 (00.0%)	2 (50%)	3 (50%)	0 (00.0%)	2 (33.3%)	5 (71.4%)	0 (00.0%)	2 (28.57%)	6 (66.66%)	0 (00.0%)	1 (11.1%)
GEN	4 (100%)	0 (00.0%)	0 (00.0%)	5 (83.3%)	0 (00.0%)	1 (16.6%)	5 (71.4%)	0 (00.0%)	2 (28.57%)	7 (77.7%)	0 (00.0%)	2 (22.2%)
INN	1 (25%)	0 (00.0%)	1 (25%)	1 (16.6%)	0 (00.0%)	0 (00.0%)	1 (14.28%)	0 (00.0%)	3 (42.85%)	0 (00.0%)	0 (00.0%)	4 (44.4%)
IPM	4 (100%)	0 (00.0%)	0 (00.0%)	5 (83.3%)	0 (00.0%)	1 (16.6%)	6 (85.71%)	0 (00.0%)	1 (14.28%)	8 (88.88%)	0 (00.0%)	0 (00.0%)
LVX	0 (00.0%)	0 (00.0%)	2 (50%)	3 (50%)	0 (00.0%)	2 (33.3%)	1 (14.28%)	0 (00.0%)	2 (28.57%)	5 (55.55%)	0 (00.0%)	1 (11.1%)
MEM	4 (100%)	0 (00.0%)	0 (00.0%)	4 (66.6%)	0 (00.0%)	2 (33.3%)	5 (71.4%)	0 (00.0%)	2 (28.57%)	8 (88.88%)	0 (00.0%)	0 (00.0%)
MXF	0 (00.0%)	0 (00.0%)	1 (25%)	2 (33.3%)	0 (00.0%)	2 (33.3%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)
NIT	1 (25%)	1 (25%)	0 (00.0%)	1 (16.6%)	0 (00.0%)	1 (16.6%)	1 (14.28%)	3 (42.85%)	3 (42.85%)	5 (55.55%)	1 (11.1%)	3 (33.3%)
SAM	0 (00.0%)	0 (00.0%)	2 (50%)	1 (16.6%)	0 (00.0%)	2 (33.3%)	3 (42.85%)	0 (00.0%)	1 (14.28%)	2 (22.2%)	0 (00.0%)	1 (11.1%)
SXT	2 (50%)	0 (00.0%)	1 (25%)	2 (33.3%)	0 (00.0%)	0 (00.0%)	2 (28.57%)	0 (00.0%)	4 (57.14%)	0 (00.0%)	0 (00.0%)	3 (33.3%)
TGC	3 (75%)	0 (00.0%)	0 (00.0%)	2 (33.3%)	0 (00.0%)	1 (16.6%)	5 (71.4%)	1 (14.28%)	1 (14.28%)	8 (88.88%)	0 (00.0%)	0 (00.0%)
TET	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	1 (16.6%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	1 (11.1%)
TZP	2 (50%)	0 (00.0%)	1 (25%)	0 (00.0%)	0 (00.0%)	3 (50%)	5 (71.4%)	0 (00.0%)	1 (14.28%)	2 (22.2%)	0 (00.0%)	2 (22.2%)
CPE	1 (25%)	0 (00.0%)	3 (75%)	2 (33.3%)	0 (00.0%)	4 (66.6%)	4 (57.14%)	0 (00.0%)	3 (42.85%)	4 (44.4%)	0 (00.0%)	5 (55.55%)
OXA	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	1 (11.1%)
CLN	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	1 (14.28%)	1 (11.1%)	0 (00.0%)	0 (00.0%)

Table 5. Antibiotic susceptibility pattern of S. aureus.

Antibiotics	*S.aureus* from Blood n=5			*S.aureus* from Sputum n=7			*S.aureus* from In.P Urine n=1			*S.aureus* from Out.P Urine n=1		
	Sensitive no. (%)	Intermediate no. (%)	Resistant no. (%)	Sensitive no. (%)	Intermediate no. (%)	Resistant no. (%)	Sensitive no. (%)	Intermediate no. (%)	Resistant no. (%)	Sensitive no. (%)	Intermediate no. (%)	Resistant no. (%)
AMC	0 (00.0%)	0 (00.0%)	1 (20%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)
AMP	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)
CIP	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	1 (100%)	0 (00.0%)	0 (00.0%)	0 (00.0%)
CLI	5 (100%)	0 (00.0%)	0 (00.0%)	7 (100%)	0 (00.0%)	0 (00.0%)	1 (100%)	0 (00.0%)	0 (00.0%)	1 (100%)	0 (00.0%)	0 (00.0%)
GEN	3 (60%)	0 (00.0%)	1 (20%)	7 (100%)	0 (00.0%)	0 (00.0%)	1 (100%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	1 (100%)
LVX	2 (40%)	0 (00.0%)	3 (60%)	7 (100%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	1 (100%)	0 (00.0%)	0 (00.0%)	1 (100%)
MXF	2 (40%)	1 (20%)	2 (40%)	7 (100%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	1 (100%)	0 (00.0%)	0 (00.0%)	1 (100%)
NIT	5 (100%)	0 (00.0%)	0 (00.0%)	7 (100%)	0 (00.0%)	0 (00.0%)	1 (100%)	0 (00.0%)	0 (00.0%)	1 (100%)	0 (00.0%)	0 (00.0%)
SXT	4 (80%)	0 (00.0%)	1 (20%)	7 (100%)	0 (00.0%)	0 (00.0%)	1 (100%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)
SAM	0 (00.0%)	0 (00.0%)	1 (20%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)
TEC	5 (100%)	0 (00.0%)	0 (00.0%)	6 (85.71%)	0 (00.0%)	0 (00.0%)	1 (100%)	0 (00.0%)	0 (00.0%)	1 (100%)	0 (00.0%)	0 (00.0%)
TET	4 (80%)	0 (00.0%)	1 (20%)	5 (71.4%)	0 (00.0%)	2 (28.57%)	1 (100%)	0 (00.0%)	0 (00.0%)	1 (100%)	0 (00.0%)	0 (00.0%)
TGC	4 (80%)	0 (00.0%)	0 (00.0%)	7 (100%)	0 (00.0%)	0 (00.0%)	1 (100%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)
PENG	0 (00.0%)	0 (00.0%)	3 (60%)	2 (28.57%)	0 (00.0%)	5 (71.4%)	0 (00.0%)	0 (00.0%)	1 (100%)	0 (00.0%)	0 (00.0%)	1 (100%)
ERY	4 (80%)	1 (20%)	0 (00.0%)	6 (85.71%)	0 (00.0%)	1 (14.28%)	1 (100%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	1 (100%)
LZD	4 (80%)	0 (00.0%)	0 (00.0%)	6 (85.71%)	0 (00.0%)	0 (00.0%)	1 (100%)	0 (00.0%)	0 (00.0%)	1 (100%)	0 (00.0%)	0 (00.0%)
OXA	1 (20%)	0 (00.0%)	3 (60%)	5 (71.4%)	0 (00.0%)	2 (28.57%)	0 (00.0%)	0 (00.0%)	1 (100%)	0 (00.0%)	0 (00.0%)	1 (100%)
VAN	5 (100%)	0 (00.0%)	0 (00.0%)	6 (85.71%)	0 (00.0%)	0 (00.0%)	1 (100%)	0 (00.0%)	0 (00.0%)	1 (100%)	0 (00.0%)	0 (00.0%)
IPM	0 (00.0%)	0 (00.0%)	0 (00.0%)	1 (14.28%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	0 (00.0%)

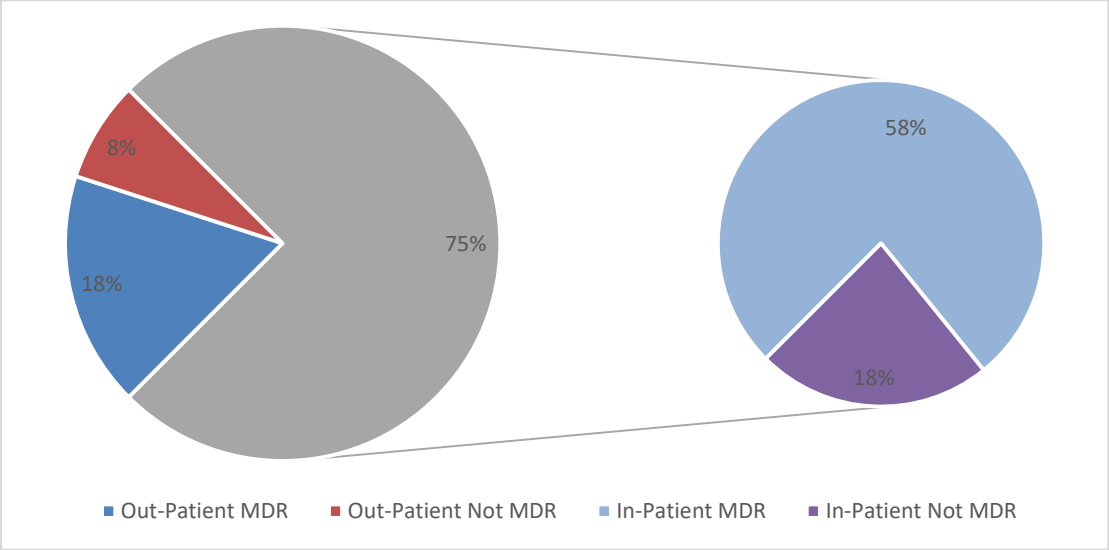

Figure 5. Percentage distribution of all subjects for MDR and not-MDR according to resistance to bacterial isolates.

Table 6. Results of GeneXpert, Xpert Carba-R Assay (Cepheid Inc, USA).

Analyte Name	Analyte Result	Probe Check Result
IMP1	NOT Detected	Pass
VIM	NOT Detected	Pass
NDM	NOT Detected	Pass
KPC	NOT Detected	Pass
OXA48	Detected	Pass

The study demonstrates a correlation between the hospitalization of the patient and acquiring a multiple drug resistance bacterial infection; in specifics it existed in individuals with weak immunity due to elderliness. Besides, the analysis of data revealed a vast difference in the gender of participants. Female participants were greater than the male with 60%.

In this study, the high prevalence of bacterial isolates was as following; K. pneumoniae 26 (21.66%), E. coli 23 (19.16%), P. aeruginosa 16 (13.33%) and S.aureus 15 (12.5%). Besides, the 120 isolates 81(67%) is gram negative and 39 (33%) is gram positive. Where in Makkah two main hospitals were discovered a ceftazidime resistance in 24.6% of E. coli, 34.4% of K. pneumoniae, and 52.7% of P. aeruginosa (Zowawi, 2016).

So, in line with the hypothesis, the nosocomial bacterial infection is highly expected to be multiple drug-resistant. Accordingly, the most multiple drug resistance bacteria were an isolate of 95-years-old who was an inpatient from the intensive care unit (ICU) ward who was admitted for almost a month. The transmission of such pathogen K. pneumonia in a hospital setting can be through direct contact with the contaminated patient and healthcare providers, catheters, or respiratory devices (Khan et al. 2015). Thus, immediate isolation of the patient was done to follow the infection control regulations.

The results investigated by (AlKhamees et al. 2018), amoxicillin and clavulanic acid complex were found to be the most two top consumed antibiotics in Saudi Arabia. However, this study found that the most resistant antibiotic was ampicillin, followed by cefepime and levofloxacin equally. On the contrary, linezolid found to have high sensitivity with 0 bacterial resistance isolates. Furthermore, the found isolate that was the most MDR of all 120 isolates was retrieved from a urine sample from the ICU ward. The isolate was identified as Carbapenem-resistant Enterobacteriaceae (CRE) K. pneumoniae. In addition, the isolate was resistant to; amikacin, amoxicillin-clavulanic acid, ampicillin, ampicillin-sulbactam, ceftazidime, ciprofloxacin, cefuroxime, cefoxitin, gentamicin, levofloxacin, nitrofurantoin,

tigecycline, meropenem, imipenem, cefepime and colistin. Hospital-acquired infections that are classified to be a CRE pose a challenging risk due to the limitations of therapeutic choices (Abdallah et al. 2018).

Carbapenem resistant Enterobacteriaceae (CRE) was described in 2017 by WHO as a critical group and need to be number one in priority of the urgency to find unconventional antibiotics for research and development (R&D) (WHO, 2017). Carbapenemase is a β-lactam-degrading enzyme, which is developed by bacteria to give it the ability to be resistant to antibiotics in the β-lactam group, such as meropenem, imipenem (Cavera et al. 2015). This study revealed antibiotic susceptibility pattern as the following; in the 120 bacterial isolates, 11(9.16%) of them were found to be sensitive, 20 (16.66%) were found to be resistant one antibiotics, 19 (15.83%) of them were found resistant to two antibiotics, and 70 (58.33%) isolates were found resistant to three or more antibiotic.

After the molecular screening of the five most common carbapenemase genes (blaIMP, bla OXA48, blaVIM, blaNDM , and blaKPC). This descriptive, cross-sectional, hospital-based study is revealing CRE isolate is positive for antimicrobial resistance gene bla OXA48. Similarly, in the Southern (Asir) province of Saudi Arabia, CRE *Klebsiella pneumoniae* was molecularly studied and found that the OXA48 resistance gene is reaching a threatening level (Al-Zahrani and Alasiri, 2018). A more plausible explanation for these findings is that non-nationals from OXA-48 endemic countries came to Saudi Arabia for work or religious purposes, such counties North African countries like Egypt, Libya, Algeria and Morocco. Also, Pakistan, India and Turkey (Al-Zahrani and Alasiri, 2018).

CONCLUSION

The most multiple drug resistance isolate the type of carbapenemase was OXA-48, *K. pneumonia*. Besides, the prevalence rates of multiple drug resistance among the isolates were high. Furthermore, the admission of patients, the immunity status of patients and the age of the patients are critical factors of acquiring a hospital infection. Also, the widespread of multiple drug resistance and the obtained rates from the study are alarming. The prescription patterns of antibiotics need to be reviewed and evaluated regularly. The limitation this study poses in the advanced molecular typing technologies, to investigate the molecular epidemiology of the isolate of this geographical region. Also, some of the methodological selections were constrained by King Abdullah Medical Complex laboratory's abilities and accessible devices.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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ETHICAL APPROVAL

Permission to conduct this study was granted by the Research and Studies Committee of Ministry of Health under the research number of (01122) and registered number with KACST (KSA: H-02-J-002).

AUTHOR CONTRIBUTIONS

AHB and RAH designed, supervised the project and reviewed the manuscript. FAK, SHA, LTA and MHA collected the samples and performed experiments and data analysis. All authors read and approved the final version.

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