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Antimicrobial resistance patterns of *Klebsiella* isolates from clinical samples in a Saudi hospital

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***Klebsiella* infection is often the leading cause of morbidity and mortality. Resistance pattern of antimicrobial susceptibility to commonly prescribed drugs were studied in *Klebsiella* isolates from a hospital in Madinah, Saudi Arabia. Clinical samples were collected from 6840 patients and screened for *Klebsiella* species over a period of 14 months. The strains were identified using microbiological and biochemical tests while the antibiotic susceptibility was performed by disk diffusion assay. Of all the positive samples isolated, only 275 samples were identified as *Klebsiella* species. Of these 66% were from males indicating that females are less vulnerable. Maximum isolates (66 to 70%) were collected from sputum and wound swabs of males. About 90% species were isolated from wound, sputum and catheter tips swabs. Antimicrobial sensitivity was studied using seventeen different antibiotics. Results revealed that antibiotic Imipenem has the highest sensitivity of 99.5% while Ampicillin revealed 100% resistance. The prescription of Imipenem antibiotic is recommended for the treatment of *Klebsiella* infections. In case of resistance to Imipenem, other antibiotics mainly Ceftazidime, Aztreonam, Ciprofloxacin, Cefoxitin and Piperacilline may be recommended. In acute cases, use of combined antimicrobial therapy may be required. Results also indicate that the intensity of *Klebsiella* infections is higher during summers (46.5%) than in winters (27.2%), while autumn (13.9%) and spring seasons (12.5%) showed the least recorded percentage. Our study will help in persistent and continuous monitoring of antimicrobial susceptibility and supplement the already available data on prevalence of antimicrobial resistance patterns of *Klebsiella*.**

Key words: *Klebsiella,* Enterobacteriaceae, antimicrobial susceptibility pattern, antibiotic resistance, multi-drug-resistance.

INTRODUCTION

Antibiotic resistance is a major problem world-wide including Saudi Arabia (Yezli et al., 2014). Several factors are responsible for the emergence of multidrug resistance, the major being wide-spread use of broad spectrum antibiotics (Ventola, 2015). The resistant bacteria can be easily transmitted among patients and from healthcare workers to patients and *vice versa* (Chan, 2012; Aly and Balkhy, 2012). Besides, the resistance pattern to antimicrobial agents has changed dramatically. With time an increase in the rate of antimicrobial resistance has been observed in several species of Gram-negative bacteria (Mahmoud et al., 2016; Chastre, 2008). The resistance rates are higher in Intensive Care Units (ICUs). It is even more alarming to see that the antibiotics used to treat bacterial infections in humans are also used in animal industry (CDCP, 2013). There is hence a need for novel and more stringent antibiotic prescription guidelines to deal with this problem.

The *Klebsiella* species are non-motile rod shaped Gram-negative bacteria with a prominent capsule. These opportunistic human commensals belong to the family Enterobacteriaceae where *Klebsiella pneumoniae* and *Klebsiella oxytoca,* are two species that are responsible for the majority of infections (Nordamann et al., 2009; Algowaihia et al., 2016). These inherently opportunistic nosocomial pathogens are known to cause pneumonia specifically in chronic alcoholics. They also cause infections in urinary tracts, gastrointestinal tracts, surgical wound infections and blood (Kamal et al., 2017). *Klebsiella* species are often resistant to many antibiotics, including cephalosporins and aminoglycosides (Manikandan and Amsath, 2013).

K. pneumoniae has been identified as an important common pathogen for nosocomial pneumonia, septicaemia and wound infections (Jadhav et al., 2012). Epidemic and endemic nosocomial infections caused by *Klebsiella* species are leading causes of morbidity and mortality. In the United States of America, *Klebsiella* is the eighth most important infectious pathogens in hospitals (Manikandan and Amsath, 2013). In Saudi Arabia, the predominant organisms that cause UTIs are Gram negative bacteria which are highly resistant to commonly used oral antibiotics (Yezli et al., 2014).

Clinical isolates of *K. pneumoniae* are generally resistant to a wider range of antibiotics, and virtually always naturally resistant to ampicillin and amoxycillin. β-lactam antimicrobial agents are the most common treatment options for such infections (Jadhav et al., 2012). Extended-spectrum β-lactamases (ESBLs) are often found in the Enterobacteriaceae family of Gram-negative bacilli, particularly *Klebsiella* species, *Escherichia coli* and *Proteus mirabilis* (Al Johani et al., 2010). *Klebsiella* species that produce *Klebsiella pneumoniae carbapenemase* (KPC) are of serious concern as they have high-level resistance against most antibiotics. These KPC enzymes efficiently hydrolyze carbapenems, as well as other β-lactam antibiotics (Paterson et al., 2005). A study comparing ICU isolates from KSA and Kuwait showed that *E. coli* and *Klebsiella* species demonstrated multidrug resistance to monobactams, cephems, and aminoglycosides (Rotimi et al., 1998).

The risk of bacterial infection increases with long term antibiotic exposure, prolonged stay in ICUs, other severe illnesses, and instrumentation or catheterization (Jiao et al., 2015). Since there may be variations in antibiotic susceptibility depending on factors like gender, location, age, etc., it is essential to understand the antimicrobial susceptibility pattern of *Klebsiella* species so that relevant measures can be taken to control the rapid spread of multidrug resistance. Hence, a descriptive hospital based study was undertaken to isolate and identify *Klebsiella* species from various clinical samples using microbiological and biochemical methods and to study gender distribution of isolates and their antimicrobial sensitivity profile.

MATERIALS AND METHODS

Sample collection

Different clinical samples such as sputum, wound swab, cerebrospinal fluid (CSF), tracheal aspirate (Tr. asp.), throat aspirate (Th. asp.), catheter Tip, pus, abdominal abscess (Abd. ab.), ear swab, peritoneal wound swab (Peri. w.s.), pleural fluid (Pler. fluid), App: Appendix (App.), bile, Urethra (Ur), and semen were collected from 6840 patients suspected of bacterial infection at King Fahd Hospital at Madinah, Saudi Arabia. Clinical samples were cultured to isolate the organisms. Demographic data such as sex of the patients was recorded prior to sample collection.

Cultivation and Identification

The clinical samples were collected according to Centers for Disease Control and Prevention Specimen Collection Guidelines (CDCP, 2013), aseptically inoculated on plates of Blood agar, Chocolate agar, Cystine-Lactose-Electrolyte-Deficient (CLED) agar and MacConkey agar (Oxoid Cambridge, UK) and incubated at 37°C for 24 h.

Identification was done based on morphological characteristics of the colonies including size, shape, colour, pigmentation and haemolytic nature.

Biochemical characteristics

Suspected *Klebsiella* colonies isolated were further identified through biochemical tests (Barrow and Felthan, 2003) using standard procedures and Phoenix automated microbiology 100 ID/AST system (Becton Dickinson Company, Sparks, Md.).

Antimicrobial susceptibility test

Susceptibility to antimicrobial agents was determined by using the disk diffusion method (Oqunshe, 2006), and Phoenix automated

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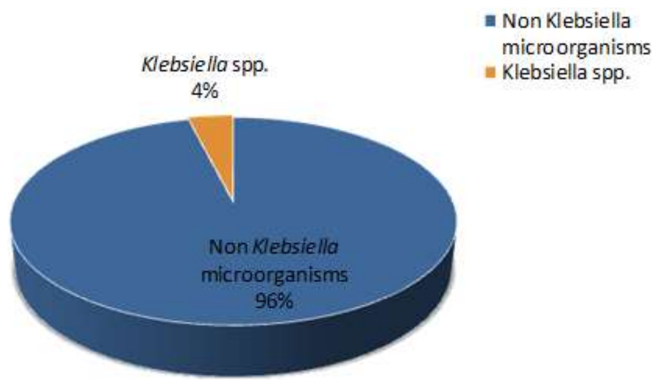

Figure 1. Percentage of *Klebsiella* spp. in comparison to other clinical isolates.

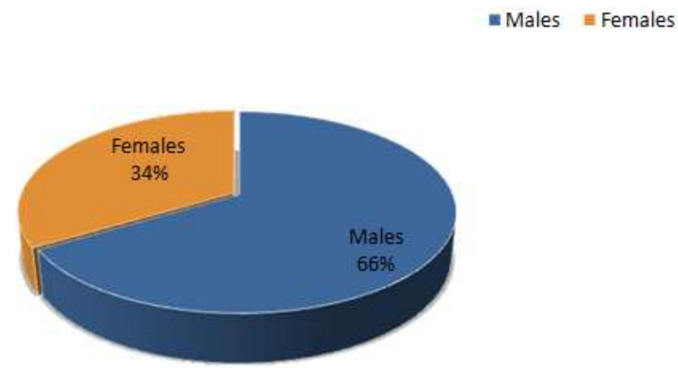

Figure 2. Percentage *Klebsiella* spp. isolates positive in males and females.

microbiology 100 ID/AST system (Becton Dickinson Company, Sparks, Md.). The following antimicrobial agents (obtained from BDH London, UK) were used: ampicillin (AP), augmentin (AUG), gentamycin (GM), cefoxitin (FOX), cephalothin (KF), cotrimoxazole (TS), amikacin (AK), ceftazidime (CAZ), aztreonam (AZT), piperacilline (PRL), imipenem (IMP), ciprofloxacin (CIP), cefpiramide (CPM), meropenem (Merop), tazobactam (Taz), colistin (Col), and nitrofurantoin (Nitro).

The inocula were prepared by growing the various *Klebsiella* strains on separate agar plates and colonies from the plates were transferred with a loop into 3 ml of normal saline. The density of these suspensions was adjusted to 0.5 McFarland standards. The surface of Muller-Hinton agar (Oxoid Cambridge, UK) plate was evenly inoculated with the organisms using a sterile swab. The swab was dipped into the suspension and pressed against the side of the test tube to remove excess fluid.

The wet swab was then used to inoculate the Muller-Hinton agar by evenly streaking across the surface. By means of a Disc Dispenser (Oxoid Cambridge, UK), the antibiotic discs were applied onto the surface of the inoculated agar and the plates were incubated overnight at 37°C. The diameter of zone of growth-inhibition observed was measured and compared to the chart provided by Clinical and Laboratory Standards Institute (CLSI, 2009).

RESULTS AND DISCUSSION

Patients, especially in ICUs are at risk of acquiring infections, most of which are associated with the use of invasive devices such as catheters and mechanical ventilators (Shulman and Ost, 2005). The ever increasing crisis of antibiotic resistance has been attributed to the overuse and misuse of antibiotics which needs to be regulated under stringent guidelines (Ventola, 2015).

The antibiotic-resistant infections place a lot of burden on the economy of any country as health care costs rise (Golkar et al., 2014). The most serious Gram-negative infections occur in health care settings are most commonly caused by Enterobacteriaceae (mostly *Klebsiella pneumoniae*) (Ventola, 2015; Yezli et al., 2014). Untreatable or difficult-to-treat infections due to Carbapenem-resistant *Enterobacteriaceae* (CRE) bacteria are on the rise among patients in medical facilities. Each year around 1.5lakh, *Enterobacteriaceae* infections occur in the U.S. which is health care-associated and approximately 600 deaths result from infections caused by the two most common types of CRE, carbapenem-resistant *Klebsiella* species and carbapenem-resistant *E. coli* (CDCP, 2013).

There are relatively few studies that deal with antibiotic use in ICUs in Saudi Arabia (Rotimi et al., 1998; Al Johani et al., 2010). In the present work, we study the antimicrobial resistance patterns of *Klebsiella* isolates from patients of a big hospital in Madinah, a city visited by lakhs of pilgrims every year. Exactly 6840 clinical samples were collected from patients suspected of bacterial infection in King Fahad Hospital during a period of 14 months. Samples were screened and in total 275 isolates were identified as positive for *Klebsiella* species, representing about 4% of all the positive samples (Figure 1). Some samples like urine, blood, ascetic fluid, nasal swabs, axilla and perineum showed complete absence of isolates. It was observed that of the positive *Klebsiella* isolates, 66% were from males while 34% from females (Figure 2) indicating that males show greater vulnerability for these infections. *Klebsiella* species were positive for nitrate reduction test; urease test, citrate utilization test, Voges-Proskauer test, lactose fermentation; and negative for H_2S gas production, oxidase test, methyl-red test, indole test, and motility.

Table 1 gives an estimation of the number of male and female samples isolated from different sources. Majority of the *Klebsiella* species (66 to 70%) were isolated from the sputum and wound swabs of male patients. A higher isolation rate from sputum and wounds has been reported in earlier studies as well (Ali and Ali, 2014; Namratha et al., 2015). Of the 126 sputum samples that were positive for *Klebsiella*, 84 were obtained from males and 42 from females. In case of wound swabs, the male to female ratio was 66:29 indicating more than double cases in men. It has been reported earlier that adult males are more susceptible to infection with *Klebsiella* species than adult females (Janda and Abbott, 2006). A similar situation was seen with catheter tips where there were 16 male samples against 12 females.

Table 1. Number of male and female samples collected from different sources.

Sex	Source of samples														
	Sp	**WS**	**CSF**	**Tr**	**Th**	**Cath**	**Pus**	**Abd**	**Ear**	**Peri**	**Pler**	**App**	**Bile**	**Ur**	**Semen**
M	84 (66.6)	66 (69.5)	3 (100)	0 (0)	4 (50)	16 (57.1)	1 (50)	1 (33.3)	1 (100)	1 (50)	2 (100)	0 (0)	0 (0)	1 (100)	1 (100)
F	42 (33.4)	29 (30.5)	0 (0)	1 (100)	4 (50)	12 (42.9)	1 (50)	2 (66.7)	0 (0)	1 (50)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)
Total	126	95	3	1	8	28	2	3	1	2	2	1	1	1	1

M: Males, F: females, Sp: sputum, WS: wound swab, CSF: cerebrospinal fluid, Tr: tracheal aspirate, Th: throat aspirate, Cath: catheter Tip, Abd: abdominal abscess, Peri: peritoneal wound swab, Pler: pleural fluid, App: appendix, Ur: urethra. percentage (%) values are given in parentheses.

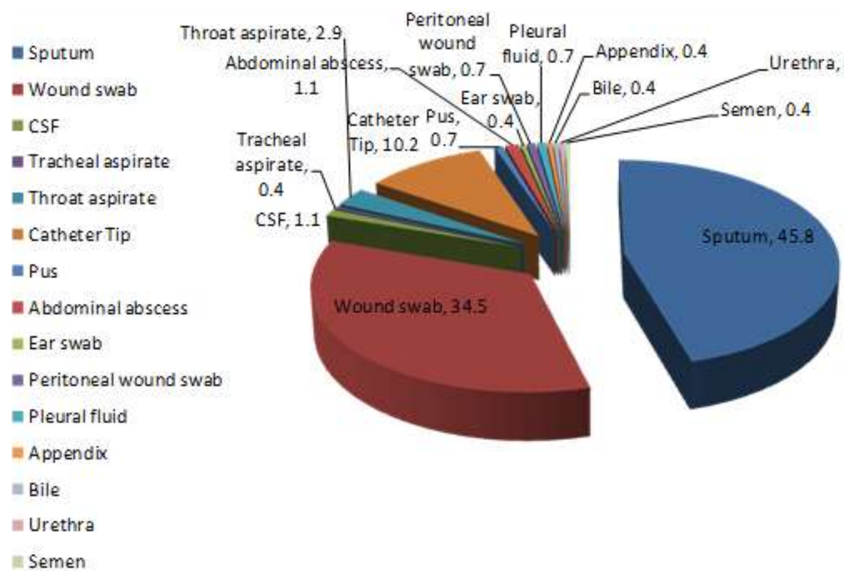

Figure 3. Percentage of *Klebsiella* samples isolated from different clinical samples.

Moreover, in a country like Saudi Arabia, where males represent a larger workforce, they are exposed more to infections, pollution, road accidents and hence more bone fractures and surgeries. All the 3 samples obtained from the cerebrospinal fluid were from males. In case of throat aspirates, the cases were from 50% males and 50% females. The numbers of the remaining samples were not enough to draw any substantial conclusion. We obtained only 1 sample each from tracheal aspirate, ear, appendix, bile, urine and semen; 2 to 3 samples from pus, abdominal abscess, peritoneal wound swab and pleural fluid.

Figure 3 shows the percentage of samples from various clinical sources that were positive for *Klebsiella* species. As mentioned earlier, the majority of isolates was from sputum and wound swabs, 45.8 and 34.5%, respectively followed by Catheter tips (10.2%). Very few strains were obtained from other clinical sources. The percentage of positive *Klebsiella* isolates from throat aspirates was 2.9% while from CSF and abdominal abscess it was 1.1%. Pus, peritoneal wound swabs and pleural fluid provided only 0.7%

Table 2. Percentage (%) of antimicrobial sensitivity pattern of *Klebsiella* samples to different antibiotics.

Antibiotic	Abbreviation	Sensitive	Resistant
Ampicillin	AP	0	100
Augmentin	AUG	66.1	33.9
Gentamycin	GM	87.3	12.4
Cefoxitin	FOX	93.7	6.3
Cephalothin	KF	49.1	50.9
Cotrimoxazole	TS	62.7	37.3
Amikacin	AK	86.6	13.4
Ceftazidime	CAZ	98.6	1.4
Aztreonam	AZT	96.6	3.4
Piperacilline	PRL	86.2	13.8
Imipenem	IMP	99.5	0.5
Ciprofloxacin	CIP	96.1	3.9
Cefpiramide	CPM	100	0
Meropenem	Merop	100	0
Tazobactam	Taz	100	0
Colistin	Col	100	0
Nitrofurantoin	Nitro	100	0

positive isolates. Tracheal aspirates, ear, appendix, bile, urethra, and semen were poor sources providing only 0.4% positive isolates each. The results here showed that the maximum number of *Klebsiella* species isolate were from sputum and wound swabs. They represented about 80% of all the clinical samples while the least number of isolates were from appendix, bile, urethra, tracheal aspirate, ear swabs and semen constituting a total of only 2.4%.

The positive samples were segregated on the basis of gender and the results have been summarized as Table 1. It was observed that in case of sputum and wound swab, around 66% and 69% samples, respectively were from males. Samples from catheter tips also included greater *Klebsiella* species from males (57%) and (42.9%) females. Another source throat aspirate revealed that male and female contribute equally, 50% each. The percentage of other samples was not enough to compare the male to female ratio (gender profile) and hence did not give reproducible results.

From the clinical samples considered here, results reveal that males are more vulnerable than females in acquiring *Klebsiella* infections which corroborates with previous reports (Shah et al., 2010; Namratha et al., 2015). As reported earlier (Janda and Abbott, 2006), this may be due to the fact that males are the major working class and being in the open, experience greater exposure to various infections.

Antimicrobial drug sensitivity was performed by disc diffusion assay using discs impregnated with seventeen antibiotics belonging to different families. These were ampicillin, augmentin, gentamycin, cefoxitin, cephalothin, cotrimoxazole, amikacin, ceftazidime, aztreonam, piperacilline, imipenem, ciprofloxacin, cefpiramide, meropenem, tazobactam, colistin, and nitrofurantoin. As shown in Table 2, Imipenem was the most effective antibiotic against *Klebsiella* species with 99.5% sensitivity followed by CAZ with 98.6% sensitivity for *Klebsiella*. The high sensitivity of Imipenem has been shown by others as well (Namratha et al., 2015; Gupta et al., 2012; Shiju et al., 2010). AZT was the third most sensitive antibiotic (96.6%) used in the present study showing high sensitivity against *Klebsiella* species. Amikacin is a fourth generation aminoglycoside which showed fairly good sensitivity, results being consistent with the previous studies (Ali and Ali, 2014; Namratha et al., 2015).

The percentage sensitivity with the remaining antibiotics in the present study was in this sequence: GM (87%) > AK (86.6%) > PRL (86.2%) > AUG (66.1%) > TS (62.7%) > KF (49.1%). The antibiotics CPM, Merop, Taz, Col and Nitro showed 100% sensitivity but as the number of strains was very low, significant conclusions could not be made. *Klebsiella* species showed 100% resistance to Ampicillin. Previous studies have shown similar resistance pattern with this drug (Jadhav et al., 2012). The chromosomally encoded β-lactamases could be responsible for this intrinsic resistance (Namratha et al., 2015). Nevertheless, it was encouraging to see that, most of the remaining antibiotics were showing high sensitivity towards *Klebsiella*. This was not the case with *Proteus* species as reported in a previous study conducted by the same group (Bahashwan and Shafey, 2013). The *Proteus* strains showed high resistance to most of the antibiotics except Imipenem which showed around 91% sensitivity. Imipenem was shown to be the most effective antimicrobial drug against Gram-negative bacteria by others (El-Tahawy, 2000).

Understanding seasonal trends in the incidence of nosocomial infections will help in improving surveillance and evaluation of infection prevention guidelines. Several reports claim that bacterial infections always peak during summers and winters (Eber et al., 2011; Anderson et al., 2008). Table 3 depicts the percentage of *Klebsiella* infections during the four different seasons. It was observed that the percentage of infections was the highest during summers (46.4%) followed by winters (27.2%). During autumn and spring, the percentages are not too high being 13.9% during autumn and 12.5% during spring. Winters are known for the outbreaks of bacterial infections especially related to the respiratory systems (Anderson et al., 2008; Eber et al., 2011). The autumn season incidentally coincides with the major pilgrimage period when a large number of pilgrims visit Madinah during Haj. But interestingly, the percentage of *Klebsiella* infections was not very high in this period in comparison to summers and winters. This can be attributed to the efforts of the health authorities who take special care to control the outbreak of bacterial and other infections. We have reported a similar pattern in case of

Table 3. Percentage (%) of *Klebsiella* infections pattern during different seasons.

Season	Percentage (%) of *Klebsiella* infections
Summer (22 June -22 September)	46.4
Autumn (23 September -21 December): Pilgrimage season	13.9
Winter (22 December -30 Mars)	27.2
Spring (21 Mars-21 June)	12.5

Proteus infection during the same period of study (Bahashwan and Shafey, 2013). Although Madinah, just like Makkah, expects a large population all year around, it is during the Haj season that there are dangers of an outbreak of epidemic.

To avoid epidemic like situation, special care is taken which could be the reason for such low percentage of infection during the pilgrimage season. The introduction and implementation of the World Health Organization (WHO) hand hygiene program by Saudi Arabia, and the conception of the Gulf Cooperation Council (GCC) Infection Control Program can be seen as a good initiative in reducing spread of resistant pathogens in healthcare units (Yezli et al., 2014).

Conclusion

Males are more vulnerable to *Klebsiella* infections than females. The most effective antibiotic with the highest sensitivity and lowest resistance was Imipenem which can hence be prescribed to patients with least reservations. All *Klebsiella* strains were resistant to Ampicillin indicating that this antibiotic should be prescribed with care.

The intensity of infections was highest during summers followed by winters. Lack of awareness, self-medication and misuse of antibiotics has aggravated multidrug resistance in microbes. There is an ardent need to formulate and adhere to new guidelines for drugs based on their sensitivity profiles.

By studying the antimicrobial resistance pattern of pathogens, we can formulate and implement better infection control policies. Developing nationwide, healthcare guidelines is essential nowadays due to increasing resistance patterns.

Furthermore, by developing a local antibiogram database we can improve the knowledge of antimicrobial resistance patterns in a particular area and will help in improving treatment strategies in Saudi Arabia. Compliance to infection prevention guidelines is essential to eliminate major outbreaks in the future.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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