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RESEARCH ARTICLE

PREVALENCE OF ANTIBIOTIC SENSITIVITY AND RESISTANCE IN *KLEBSIELLA PNEUMONIA* ISOLATES FROM OMANI PATIENTS

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ABSTRACT

Klebsiella pneumoniae is currently regarded as one of the most important opportunistic pathogen causing nosocomial and community infections naturally resistant to amino-penicillins and carboxy-penicillins due to extended spectrum beta-lactamase producing genes, which are associated with multidrug resistance. The main purpose of the present work was to retrospectively study the pattern of antibiotic resistance and sensitivity in Omani patients testing *K. pneumoniae* positive from 2010 to 2013 and also to know category of resistant antibiotics, common sensitive and resistance antibiotics. This was a chart review study carried out retrospectively from the microbiology laboratory data and epidemiology records of 85 Omani cases having *K. pneumonia*e infection. The laboratorial records covers type of antibiotics used, whether the antibiotic sensitive or resistance, name of organism, type of specimen and the year. The resistance rate of patient isolates of *K. pneumoniae* to ampicillin remains high 99%; augmentin, 84%; cephalosporins ranged between 42 - 81%; ciprofloxacin, 28%; trimethoprim-sulfamethoxazole, 37%; amikacin, 32%; gentamicin, 32%; pipracillin, 39% of patients. The most sensitive antibiotic for *K. pneumoniae* was imipenem in 96% of cases. In conclusion, we have seen increased rates of *K. pneumoniae* incidence overtime and resistance rates to most of antibiotics tested except imipenem. Therefore, we recommend continued surveillance of antibiotic resistance and selective use of antibiotics to control rapid increase in antimicrobial resistance especially against *K. pneumoniae* to avoid its outbreak.

INTRODUCTION:

Klebsiella pneumoniae is a gram negative, anaerobic and nonflagellated bacilli belongs to the family Enterobacteriaceae[1]. It exists in the natural environment in surface water, soil, on plant and colonize the mucosa of mammals. *K. pneumoniae* is currently regarded as one of the most important opportunistic pathogen causing nosocomial and community infections, particularly among immunocompromised individuals including elders and neonates leading to increased rates in hospitalisation[2]. The incidence of community-acquired pneumonia is recognised over 100 years ago. It is the second most common cause of Gram-negative bacteraemia and urinary tract infections[3]. However, its role as a common healthcare-associated pathogen causing infections of the urinary tract, bloodstream, pneumonia, liver abscess, meningitis and intra-abdominal infections has become exceedingly common[4-5]. The incidence is highest in the summer months due to higher colonization rates[6]. As a nosocomial pathogen, it produces 3% to 17% of all bacterial infections leading to fatality varying from 18% to 68%[6-7].

K. pneumoniae is naturally resistant to amino-penicillins (Ampicillin and Amoxicillin) and carboxy-penicillins (Carbenicillin) due to the production of SHV-1, a potent penicillinase. *K pneumoniae* frequently contains plasmid-encoded extended spectrum beta-lactamase (ESBL)-producing genes, which are associated with multidrug resistance[4]. The exact cause of developing resistance is not well established however, many factors include capsule and hypermucoviscosity, lipopolysaccharides, adhesins, iron acquisition systems, serum resistance, transposons, along with the broad utilizations of antibiotics and biofilm formation[2]. ESBL-producer infections were usually associated with old age, urinary tract anomaly or catheter use, previous hospitalization, and recent exposure to antimicrobial agents[8]. Currently, more than 30 plasmids ranging from 3 kb to 270 kb have been identified. Moreover, these plasmids often carry genes encoding co-resistance to other antibiotics such as

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aminoglycosides, tetracyclines, chloramphenicol, fluoroquinolones and sulfamethoxazole–trimethoprim, to name a few[9].

Carbapenem resistance is conferred through the expression of carbapenemases, encoded by mobile genes facilitating rapid horizontal spread[6]. *K. pneumoniae* carbapenemase (KPC) enzymes belong to molecular class A carbapenemases which also include SME (Serratia marcescens enzyme), NMC/IMI (non-metallo-carbapenemase/imipenem hydrolysing b-lactamase) and GES (Guiana extended spectrum) enzymes. Class A enzymes originated in the USA but have spread globally are inhibited by clavulanate and can hydrolyse penicillins and cephalosporins more efficiently than carbapenems. Class B enzymes epicentred in India with growing international dissemination are metallo-b-lactamases (MBLs) such as VIM (Verona imipenemase), IMP (imipenemase), GIM (German imipenemase), SIM (Seoul imipenemase) and NDM (New Delhi metallo-b-lactamase). MBLs can hydrolyse carbapenems and most other b-lactams, but not aztreonam, and are inhibited by chelating agents such as EDTA or dipicolinic acid (DPA). Class D carbapenemases, was first described in Turkey but has proliferated across the Middle East and North Africa with outbreaks in Europe the OXA b-lactamases, hydrolyse carbapenems weakly, but are poorly inhibited by clavulanic acid. In the Arabian Peninsula, NDM and OXA-48 have been reported in Oman, the United Arab Emirates and Kuwait[10-11].

The emergence of strains resistant to carbapenems has left only limited treatment options; that is, tigecycline, colistin, aminoglycosides and, only in some rare cases, to carbapenems per se[12]. Due to the overuse of the cephalosporins and aminoglycosides, several outbreaks caused by *K. pneumoniae* have been recorded in many countries. Resistance is continuously increasing and spreading through more antibiotic pressure and travel[4]. It is responsible for therapeutic failure, increase hospital costs, considerable mortality and morbidity thereby challenging clinical microbiologists, pharmacologists and clinicians[13]. Surveillance of antibiotic resistance and susceptibility is very crucial to control antibiotic resistance as it is evident that the use or overuse of antibiotics may affect efficacy of a particular antibiotics and emergence of resistance in a particular organism highlighting the need to introduce control measures to overcome such condition[14].

There are hardly any published scientific reports on the prevalence of antibiotic resistance to *K. pneumoniae* in Omani patients. The main aim of the present work was to retrospectively study the pattern of antibiotic resistance and sensitivity in patients testing *K. pneumoniae* positive at the Central Laboratorial Investigation, Muscat and Ibri Regional Hospital, Ibri from 2010 to 2013 in Omani patients and also to know category of resistant antibiotics, common sensitive and resistance antibiotics.

Material and Methods:

K. pneumoniae isolates were identified retrospectively from the microbiology laboratory data and epidemiology records available at Central Laboratorial Investigation, Muscat and Ibra Regional Hospital Lab from 2008 to 2013. Targeted population was 100 however; we included only 85 Omani cases having *K. pneumonia*e infection. This is a retrospective chart review describing the outcomes of 85 cases of *K. pneumonia*e infections. The laboratorial records covers type of antibiotics used, whether the antibiotic sensitive or resistance, name of organism, type of specimen and the year.

Medical Ethics:

Study was conducted according to the guidelines and ethics of selected centers. Accordingly, the identity of the patient and their related information are kept confidential. Study was conducted only after the official permission from the centers.

Data Analysis:

The data collected was directly entered into Statistical Package for the Social Sciences (SPSS version 19.0; SPSS, Chicago, IL, USA). The frequency of antibiotics used were calculated in terms of percentage.

RESULTS:

Sources of specimens collected for testing:

Total 100 isolates of *K. pneumoniae* were identified in the study period, 15 isolates of which were excluded due to confusion on nationality of patients from whom the specimen collected. The remaining 85 isolates constituted the study sample and were subjected to further analysis. As shown in figure 1, most of the isolates were obtained from urine specimen (63%) followed by swab (18%), esophagotracheal (11%), sputum (5%) and blood (3%). Figure 2, shows the total number of cultures tested positive for *K. pneumoniae* were highest (60%) in 2013. Increase in number of positive testing for *K. pneumoniae* from 3, 14, 17 and 51 cases in 2008, 2009, 2010 and 2013 respectively is an indirect reflection of trends of increased *K. pneumoniae* cases in Omani patients.

Frequency of commonly tested antibiotics for resistance and sensitivity:

Figure 3, illustrates that ampicillin, augmentin, and ceftazidime were tested almost for 92% *K. pneumoniae* cultures for sensitivity and resistance. 80% of the time cotrimoxazole, ciprofloxacin and gentamycin were used. Third most commonly used antibiotics for testing

sensitivity and resistance were amikacin, cefuroxime, ceftriaxone in almost 49% of cultures.

Frequency of *K. pneumoniae* resistance and sensitivity to antibiotics:

As shown in figure 4 and 5, the resistance rate of patient isolates of *K. pneumoniae* to ampicillin remains high 99%, but its combination with clavulanic acid reduced resistance to 84%. However, it is very important to note that only 5.8% of specimens shown clear and significant inhibition. Resistance to cephalosporin remained low, and ranged between 81% for cefotaxime, 80% for cefuroxime, 42% for ceftazidime and 77% ceftriaxone. The resistance rate to ciprofloxacin was 28%; trimethoprim-sulfamethoxazole (cotrimoxazole) was 37%; amikacin resistance remained low to 32%; gentamicin in 32% of samples; pipracillin was resistant in 39% of patients. The most sensitive antibiotic for *K. pneumoniae* was imipenem in 96% of cases.

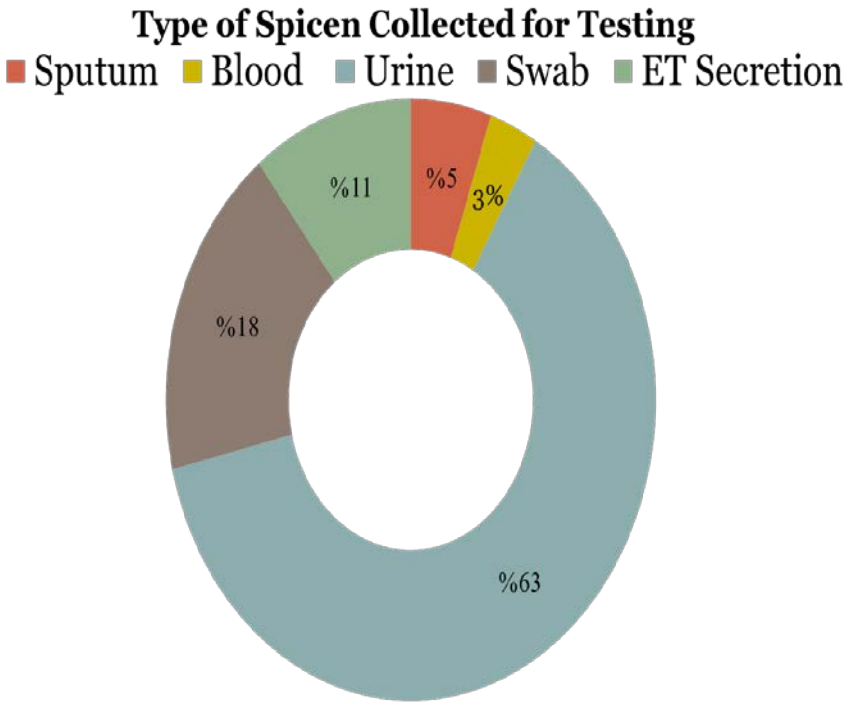

Figure 1:

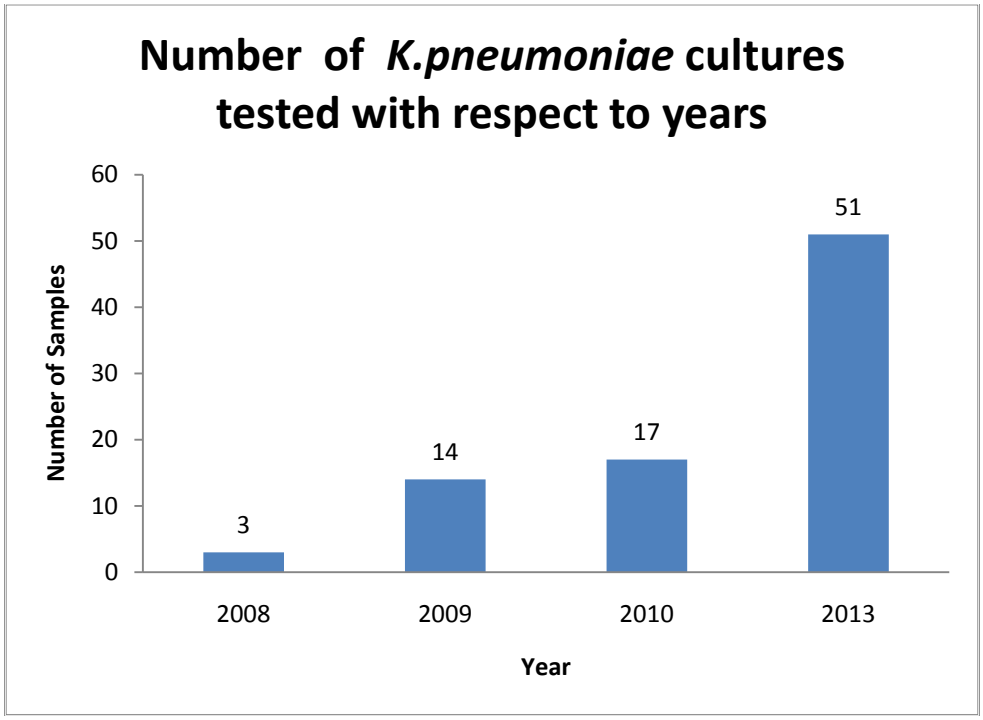

Figure 2:

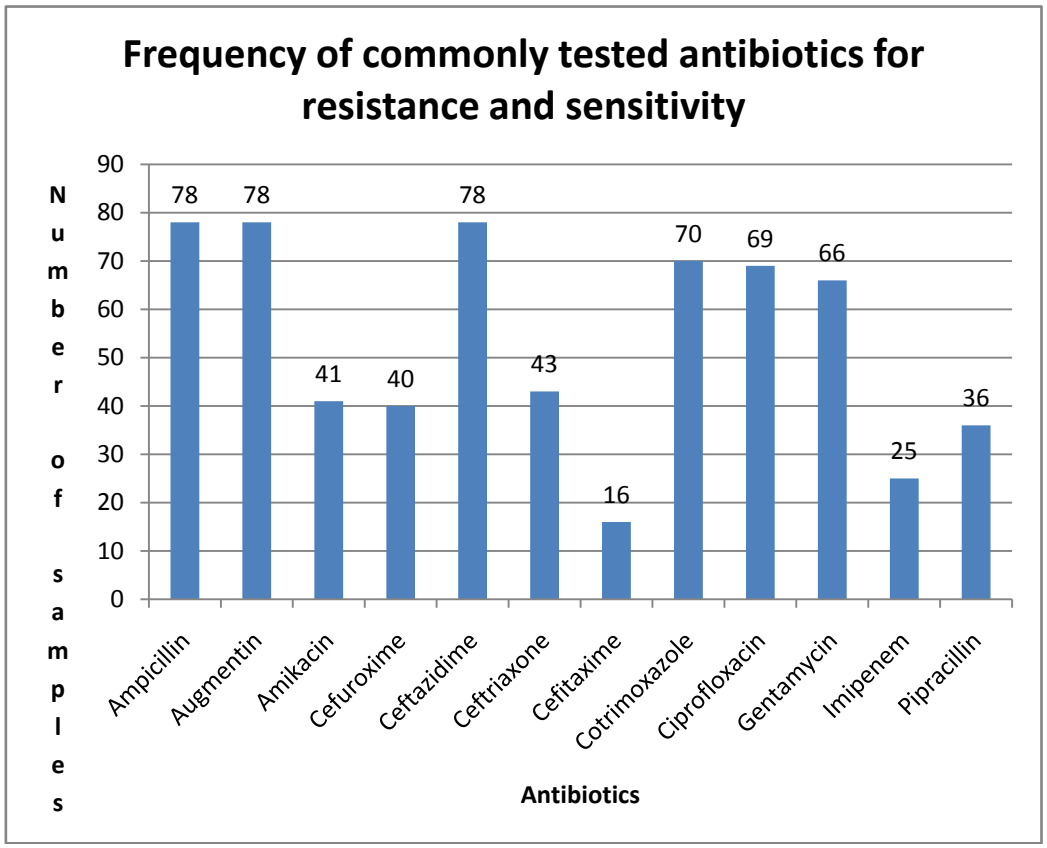

Figure 3:

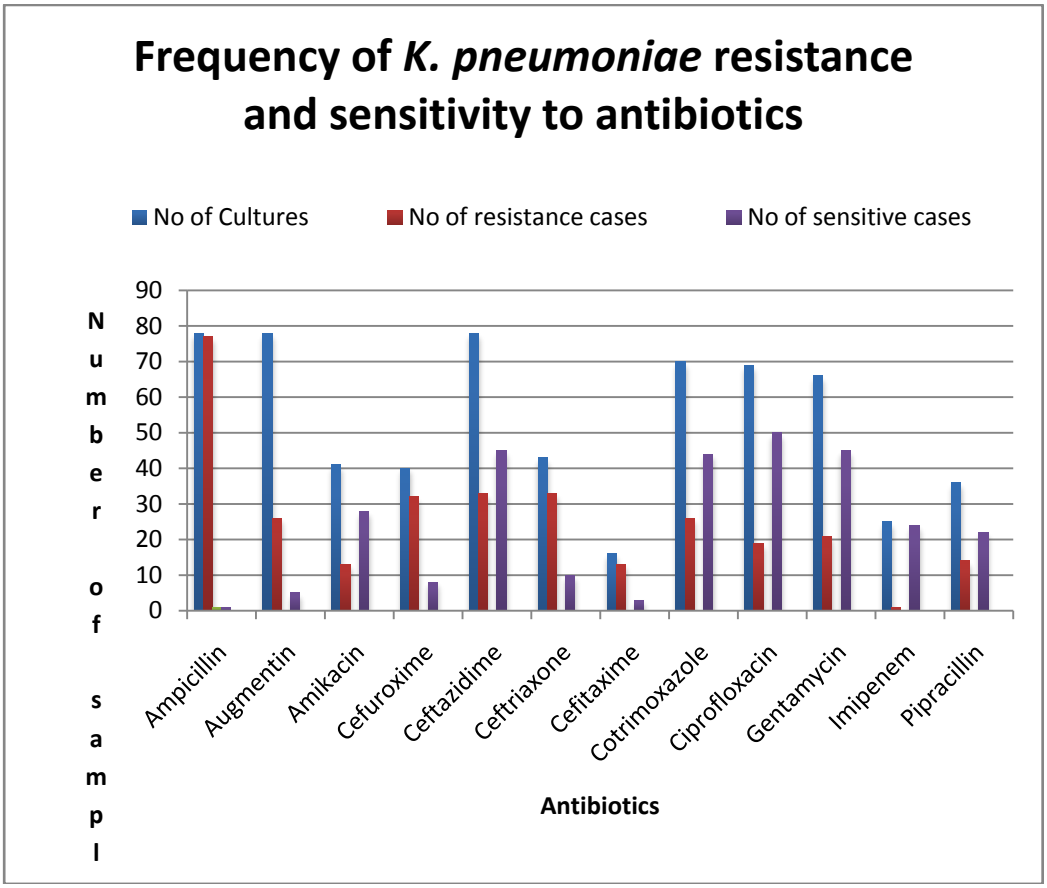

Figure 4:

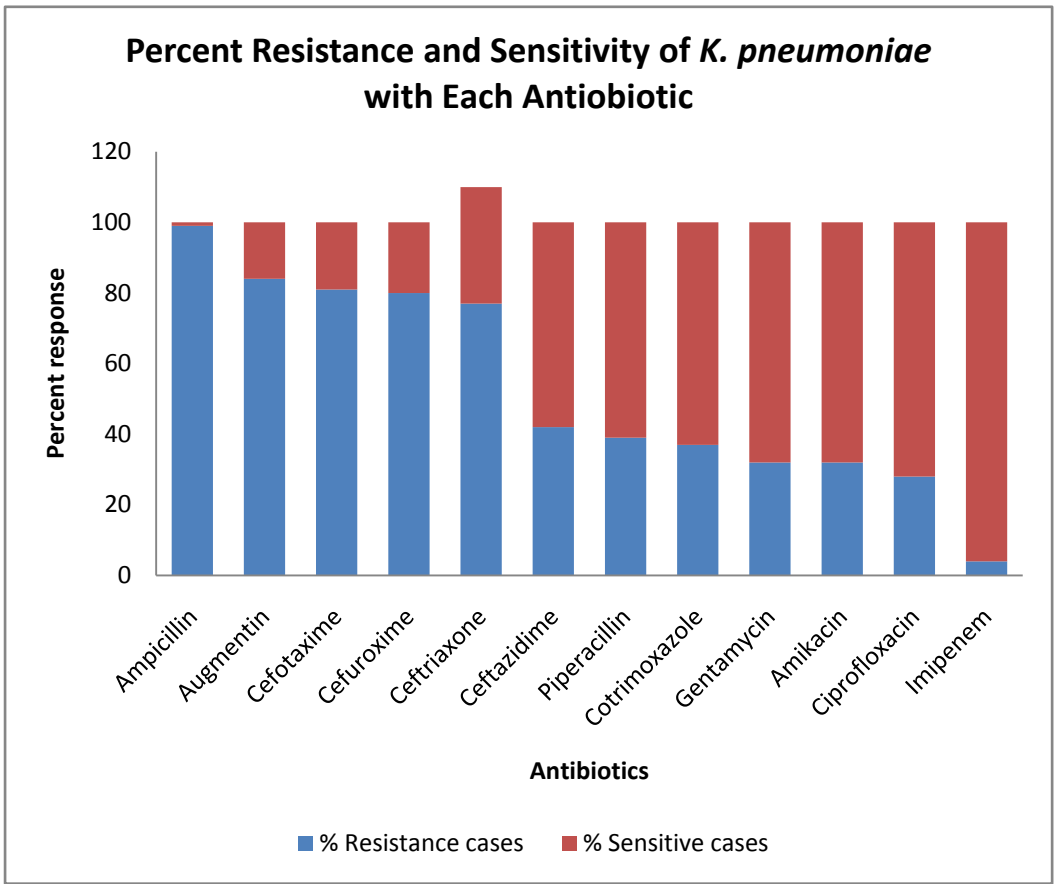

Figure 5:

DISCUSSION:

Carbapenemase-positive gram-negative organisms have spread worldwide; however, their local epidemiology and clinical characteristics vary. In Israel, Greece, and Colombia it remains endemic, whereas in Australia, New Zealand, and Canada it is mainly due to population migration. Some countries are affected due to clonal expansion others have experienced a plasmid expansion[15]. Present study was a small but a significant effort in Oman to know the prevalence of antibiotics resistance and susceptibility of *K. pneumoniae* isolates obtained from infected Omani patients. This type of regional and local antibiotic resistance studies provide an insight into the global problem of antibiotic resistance. It will also assists in selecting an appropriate antibiotics by clinicians, microbiologists, pharmacists and pharmacologists when they are indecisive.

In our study, urinary specimens were most frequently used for testing antibiotic sensitivity and resistance to *K. pneumoniae* most probably due to the fact that urinary tract remains the most common site for infection[3]. The second most common site of specimen collection was the swab and endotracheal secretion as it is already known that *K. pneumoniae* is associated with aspiration pneumonia in many elderly patients[16]. In this study, only few cases involve collection of blood cultures in reciprocal to the report from Kuala Lumpur which identifies the importance for early detection of antibiotic susceptibility and commencement of appropriate antibiotics[17].

Similar to the community based study, our results indicates that the *K. pneumoniae* has multidrug resistance to ampicillin, augmentin, piperacillin, cotrimoxazole, third and fourth-generation cephalosporins, fluoroquinolones and aminoglycosides[18-19]. The most resistant and susceptible antibiotics identified in Iran were ampicillin and imipenem, respectively which is more specifically replicated in our study[13]. The rate of prevalence of *K pneumoniae* resistance to ampicillin was 99% similar to the study from Hillbrow Hospital, Johannesburg (100%)[20] but higher than Portuguese health institutions report of 89%[21]. Level of resistance was reduced by the addition of beta-lactamase inhibitor clavulanic acid (Augmentin) from 99% to 84% in line with 80% resistance reported from Pakistan[6]. The underlying reason is due to the presence of beta-lactamase enzyme responsible for destabilization and hydrolysis of beta-lactam antibiotics.

In our study, *K. pneumoniae* has shown 32% resistance to amikacin and gentamycin almost similar to 37%

resistance in Spain[22] unlike previously reported susceptibility to amikacin in a district general hospital in the UK[11]. Proposed underlying mechanism was the presence of 16S rRNA methylases and 3'-phosphotransferase[19]. The resistance rates to trimethoprim- sulfamethoxazole was 37% higher than Saudi Arabia (9.6%)[14] and in line with Portuguese (31%)[21].

Our results revealed that 39% *K. pneumoniae* isolates were resistant to piparacillin higher than 26.9% reported in a recent past due to an enzyme DHA-1 and expression of multiple beta-lactamases within a single organism[28]. A decade ago, Cefotaxime and Piperacillin/tazobactam were the most active penicillin and cephalosporins against *K. pneumoniae* (95.6% and 82.9% of isolates susceptible respectively) [29].

We found that resistance to cephalosporins is varying from 42% to 81% similar to the earlier studies reporting more than 50% to 74.1%[16, 24]. These resistance profiles were proposed to be due to expression of different enzymes such as extended-spectrum beta-lactamases, over expression of efflux systems[16], overuse of third-generation cephalosporins[23] may also be due to production of DTL enzymes[24].

In Turkey, *K. pneumoniae* isolates were identified with high-level of imepenem resistance[25]. In Japan, the plasmids responsible for resistance were identified and accordingly these isolates showing higher resistance to β-lactams except imipenem were designated as ISMRK (imipenem-susceptible but meropenem-resistant Klebsiella) [26]. A study performed in the Czech Republic evaluated the *K. pneumoniae* infections in which meropenem was the only susceptible antimicrobial agent[27]. Here, in our study *K. pneumoniae* strains remained only susceptible to imipenem in most of the cases and remain as the only reliable alternative treatment. Most probably is due to its higher stability against Beta-lactamase hydrolysis and retained susceptibility of ESBL producers. This study supports the earlier report from Saudi Arabia and underlines the need to be always vigilant and reminds the physicians, infectiologists, hygienists, and bacteriologists of the need for a multidisciplinary approach to multi drug resistance to limit as well as its emergence and diffusion[14, 28].

Our study results are in line with results reported elsewhere, but unusually different. Based on these limited sample size it is difficult to explain trends and mechanism of *K. pneumoniae* infection and resistance to antibiotics. Based on literatures it is evident that increased antimicrobial resistance in *K. pneumoniae* is related to the excessive and erratic antibiotic usage. However, replacement of only the antibiotic class appears to be insufficient to control antibiotic resistance. Therefore, it is recommended to decrease overall antibiotic usage especially in highly endemic situations[28].

Limitations of our study were that we had no data regarding antibiotic usage, we have not considered distribution of patients according to region, gender, age, site of specimen collection, multidrug resistance, risk factors, migration history, Co-resistance, whether community or hospital acquired, and does not perform ESBL production testing. Since our study was conducted retrospectively from limited number of patient files at only two centers and therefore, there may be selection bias, which limits the general application of the study results to other areas. We recommend the readers to consider these points while data usage and for future study.

CONCLUSION:

In conclusion, we have seen increased rates of *K. pneumoniae* incidence overtime and resistance rates to ampicillin, augmentin, piperacillin, cefuroxime, ceftazidime, ceftriaxone, cefotaxime, cotrimoxazole, amikacin and gentamycin. Our study also revealed that Imipenem is the only most susceptible antibiotic. These results are an indication of possible multidrug resistance strains of *K. pneumonia* in Oman. Therefore, we recommend continued surveillance of antibiotic resistance and selective use of antibiotics to control rapid increase in antimicrobial resistance especially against *K. pneumoniae* to avoid its outbreak.

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