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Identification of *Klebsiella pneumoniae* and *Klebsiella oxytoca* in urine specimens from a laboratory in Karachi

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ABSTRACT

This study aims to identify *Klebsiella pneumoniae* and *Klebsiella oxytoca* in urine specimens. The study was carried out in the Dr. Essa Laboratory & Diagnostic Centre, Karachi, Pakistan from January 2014 to December 2014. A total number of 470 urine samples from patients of January 2014 to December 2014 were included in the study from all branches of Dr. Essa Laboratory & Diagnostic Centre. Isolates of *K. pneumoniae* and *K. oxytoca* were identified by their morphological and biochemical characteristics. All isolates were subjected to antibiotic sensitivity testing by modified Kirby bauer disc diffusion method. The number of *K. pneumoniae* isolates was 240 while *K. oxytoca* isolates was 230 from 470 urine samples. Majority of the strains were sensitive to Amikacin. In conclusion, the present study exposed the *K. pneumoniae* and *K. oxytoca* strains in patients and their tendency towards antibiotic resistance. The objective of the present study was to know the antibiotic sensitivity pattern of *K. pneumoniae* and *K. oxytoca* strains isolated from urine samples, where we found most of the strains isolated were sensitive to Amikacin and Imipenem.

Keywords: *Klebsiella pneumoniae*, *Klebsiella oxytoca*, UTI, Amikacin, Imipenem.

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INTRODUCTION

Klebsiella spp. is an increasingly significant opportunistic pathogen associated with severe infections such as pneumonia, septicemia, neonatal sepsis, wound infections and urinary tract infections (Brisse and Verhoef, 2001; Conceic et al., 2005). *Klebsiella* spp. is an opportunistic pathogen that cause a broad range of infections in male (Brisse and Verhoef, 2001). According to one report, 8% of all nosocomial bacterial infections in the United States and in Europe are caused by *Klebsiellae*, mostly through *Klebsiella oxytoca* (Darby et al., 2014) and *Klebsiella pneumoniae.*

K. oxytoca is a non-motile, gram-negative rod-shaped bacterium which belongs to the family Enterobacteriaceae. *K. oxytoca* is everywhere in the environment and can be cultured from the skin, mucous membranes, oropharynx, intestines and variety of tissues of healthy humans (Darby et al., 2014).

The ecological habitats of *Klebsiella* include surface water, soils, plants and sewage. In humans, *K. pneumoniae* can be current in the naso pharynx and in the intestinal tract.

K. pneumoniae and *K. oxytoca* exhibit an elevated d egree of genetic heterogeneity, as was established by O-antigen variation (Podschun and Ullmann, 1998), protein electrophoretic profiling (Mizuta et al., 1983), multilocus enzyme electro-phoresis (Ferragut et al., 1989), ribotyping (Combe et al., 1994), randomly amplified polymorphic DNA (RAPD) analysis (Bingen et al., 1993), pulsed-field gel electrophoresis (Wong et al., 1994) and diversity of β - lactamase genes (Poh et al., 1993). The association among genetic virulence, variability and transmissibility of *Klebsiella* strains is not understood, but there is an obvious proof for differential behavior of *Klebsiella* strains.

Within the genus *Klebsiella*, *K. pneumoniae* and *K. oxytoca* account for the majority of clinical isolates,

differing biochemically in that *K. oxytoca* is capable to produce indole from tryptophan.

Some mechanisms of the cause of antibiotic associated hemorrhagic colitis have been recommended, including allergic reaction, mucosal ischemia (Fournier et al., 1996), and infection with *K. oxytoca*. *K. oxytoca* strains isolated from patients with antibiotic connected hemorrhagic colitis and half of the strains isolated from healthy subjects have been shown to produce a cytotoxin that is able of inducing cell death in various epithelial cell cultures (Yonei et al., 1996).

The accurate identification of *Klebsiella* species is not plainly accomplished in most clinical microbiology laboratories, for the reason that several species share a similar biochemical profile. Studies have shown that a proportion of isolates classified as *K. pneumoniae* could actually be *Raoultella planticola* (Higaki et al., 1990; Monnet et al., 1991), *Raoultella terrigena* (Higaki et al., 1990; Westbrook et al., 2000), or *K. variicola* (Podschun and Ullmann, 1992; Brisse and Duijkeren, 2005) and some of the isolates classified as *K. oxytoca* actually could be *R. planticola* (Rosenblueth et al., 2004; Monnet et al., 1991). Despite that, most clinical isolates classified as *Klebsiella* spp. belong to the *K. pneumonia* (indole-negative isolates) or *K. oxytoca* (indole positive isolates) species (Westbrook et al., 2000; Hansen et al., 2004; Liu et al., 1997; Podschun and Ullmann, 1998). Though, *K. variicola*, *R. planticola*, and *R. terrigena*, in addition to *K. pneumoniae*, can explain a negative indole reaction (Podschun and Ullmann, 1992; Watanakunakorn and Jura, 1991; Brisse and Duijkeren, 2005; Monnet et al., 1991). On the other hand, *R. planticola* can expose a positive indole reaction, in adding to *K. oxytoca* (Rosenblueth et al., 2004; Monnet et al., 1991).

MATERIALS AND METHODS

Bacterial isolates from January 2014 to December 2014, 470 clinical *Klebsiella* isolates were obtained from routine patient's urine samples coming to all branches of Dr. Essa Laboratory & Diagnostic Centre, in different areas of Karachi, Pakistan.

Demographic data (like age, sex) of the patients was recorded prior to sample collection. There were no ethical matters concerned with this study, as results from routine laboratory diagnosis of clinical samples constituted the data for analysis; no particular identifiable group of patients were involved.

A total of 470 mid–stream clean catch urine samples urine were included and isolates were inoculated on EMB & CLED media (Oxoid Ltd, Basingstoke, UK) and incubated aerobically at 37°C for 24 h. After 24 h incubation, the cultures plates were examined for growth of *Klebsiella* spp. Morphology of *K. pneumoniae* identified was large, dome shaped. In gram staining, gram negative, short, plump, straight rods were seen. The biochemical characters identified were negative indole test, negative methyl red test, positive voges - proskauer test, positive citrate utilization test, positive urease test, acid and abundant gas production from glucose, lactose, sucrose, maltose and mannitol sugar fermentation tests.

Antibacterial susceptibility testing of *K. pneumoniae* and *K. oxytoca* to commonly used antibiotics was determined on Mueller-Hinton agar (Oxoid Ltd, Basingstoke, UK) by the Kirby – Bauer disc diffusion sensitivity technique using amikacin, gentamicin, tobramycin, amoxil / ampicillin, augmentin, piperacillin + tazobactam, ciprofloxacin, enoxacin, nalidixic acid, pipemidic acid, sparfloxacin, cefuroxime, ceflxime, cefotaxime, ceftazidime, ceftriaxone, cefoperazone + sulbactam, imipenem, doxycycline, chloramphenicol, cotrimoxazole, lincomycin and nitrofurantoin discs. Inoculated plates were incubated at 37°C for 18 to 24 h after which the inhibition zone diameters for each antibiotic were interpreted, as Resistant and Sensitive. The zone diameters were estimated using calipers and according to the method recommended using the National Committee for Clinical Laboratory Standard (NCCLS, 1987) and the WHO.

RESULTS

Out of a total of 470 isolates of *K. pneumoniae* and *K. oxytoca* studied. *K. pneumoniae* isolates was 240 (57.4%) while the number of *K. oxytoca* isolates was 230 (42.5%) from 470 urine samples.

Table 1 shows antibiotic susceptibility of *K. pneumoniae* isolates, where majority of the isolates were resistance to Amikacin (4.0%) and Imipenem (2.0%). Table 2 shows that antibiotic susceptibility of 230 *K. oxytoca* isolates, majority of the strains isolated were also resistant to Amikacin (0.5%).

DISCUSSION

Klebsiella spp. are the most frequent inhabitants among the intestinal micro flora causing infections such as cystitis, pyelonephritis, septicemia, pneumonia, peritonitis, meningitis, and device-associated infections. They can spread easily among humans and cause community acquired infections (Sarathbabu et al., 2012). *Klebsiella* spp. is difficult to identify and are often misclassified in clinical microbiology laboratories (Westbrook et al., 2000; Monnet, 1994; Rosenblueth et al., 2004; Westbrook et al., 2000; Monnet et al., 1991).

However, the correct identification of *Klebsiella* isolates is important for improved taxonomic and molecular epidemiologic characterization of this bacterial group. In the present study, we characterized a collection of 470 recent *Klebsiella* isolates.

According to one study *Klebsiella* species were recovered from 205 clinical samples with K. pneumonia being the highest recovered species (74.1%), followed by *K. oxytoca* (24.4%) (Acheampong and Boamponsem, 2011).

In this study, *K. pneumoniae* isolates was 240 (57.4 %) while the number of *K. oxytoca* isolates was 230 (42.5%) from 470 urine samples.

In the present study, greater part of the strains isolated was sensitive to amikacin. The percentage of resistant to Amikacin was 4.0% in *K. pneumoniae*, while 0.5% was resistant in *K. oxytoca* samples. Amikacin and Imipenem are the most effective antibiotics being 100% active against *K. pneumoniae* strains; this may be that these antibiotics have not been extensively used to cause

Table 1. Antibiotic susceptibility of *Klebsiella pneumoniae* isolates evaluated in the present study.

Antibiotic	Percentage of *Klebsiella pneumoniae* showing susceptibility to commonly used antimicrobials
	Resistant
Amoxil / Ampicillin	97.0
Doxycycline	93.6
Nalidixic acid	86.5
Chloramphenicol	60.0
Nitrofurantoin	60.0
Pipemidic acid	58.3
Cefoperazone + Sulbactam	49.0
Lincomycin	48.0
Cotrimoxazole	44.6
Cefuroxime	43.4
Ceflxime	38.2
Augmentin	37.4
Sparfloxacin	32.0
Enoxacin	29.4
Ceftriaxone	27.0
Cefotaxime	25.8
Ceftazidime	23.6
Ciprofloxacin	21.0
Tobramycin	18.0
Gentamicin	16.5
Piperacillin + Tazobactam	7.0
Amikacin	4.0
Imipenem	2.0

Table 2. Antibiotic susceptibility of *Klebsiella oxytoca* isolates evaluated in the present study.

Antibiotic	Percentage of *Klebsiella oxytoca* showing susceptibility to commonly used antimicrobials
	Resistant
Doxycycline	96.4
Amoxil / Ampicillin	94.8
Nalidixic acid	90.4
Ciprofloxacin	86.6
Pipemidic acid	83.3
Cotrimoxazole	71.3
Chloramphenicol	65.5
Nitrofurantoin	58.2
Lincomycin	55.7
Ceflxime	54.3
Cefuroxime	53.6
Sparfloxacin	46.8
Enoxacin	46.3
Cefoperazone + Sulbactam	38.6
Augmentin	38.3
Cefotaxime	37.5
Ceftriaxone	34.4
Tobramycin	33.0
Gentamicin	32.0

Table 2. Continues.

Ceftazidime	21.7
Imipenem	4.0
Piperacillin + Tazobactam	3.0
Amikacin	0.5

resistance developing against them by acquiring resistant genes (Monnet, 1994).

Carmeli score have not been performed due to some study limitations. Carmeli score, which is calculated based on key facts of the patient's medical history and often used as a readily available instrument for assessing the expected sensitivity to antibiotics in bacterial infections nowadays increasingly used in clinical practice (Streinu-Cercel, 2013). Susceptibility tests have not performed for colistin, which is a major antimicrobial agent (Streinu-Cercel, 2014).

The conclusion of this study of *K. oxytoca* and *K. pneumoniae* emphasizes the need for consideration of the *K*. *oxytoca* which can cause some severe infections, septic shocks and bacteremias in immunocompromised individuals including recipients of different forms of HSCT. Careful clinical assessment, taking sufficient investigations and administration of suitable antimicrobial therapy are necessary not only to control these infections but also to prevent additional complications.

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