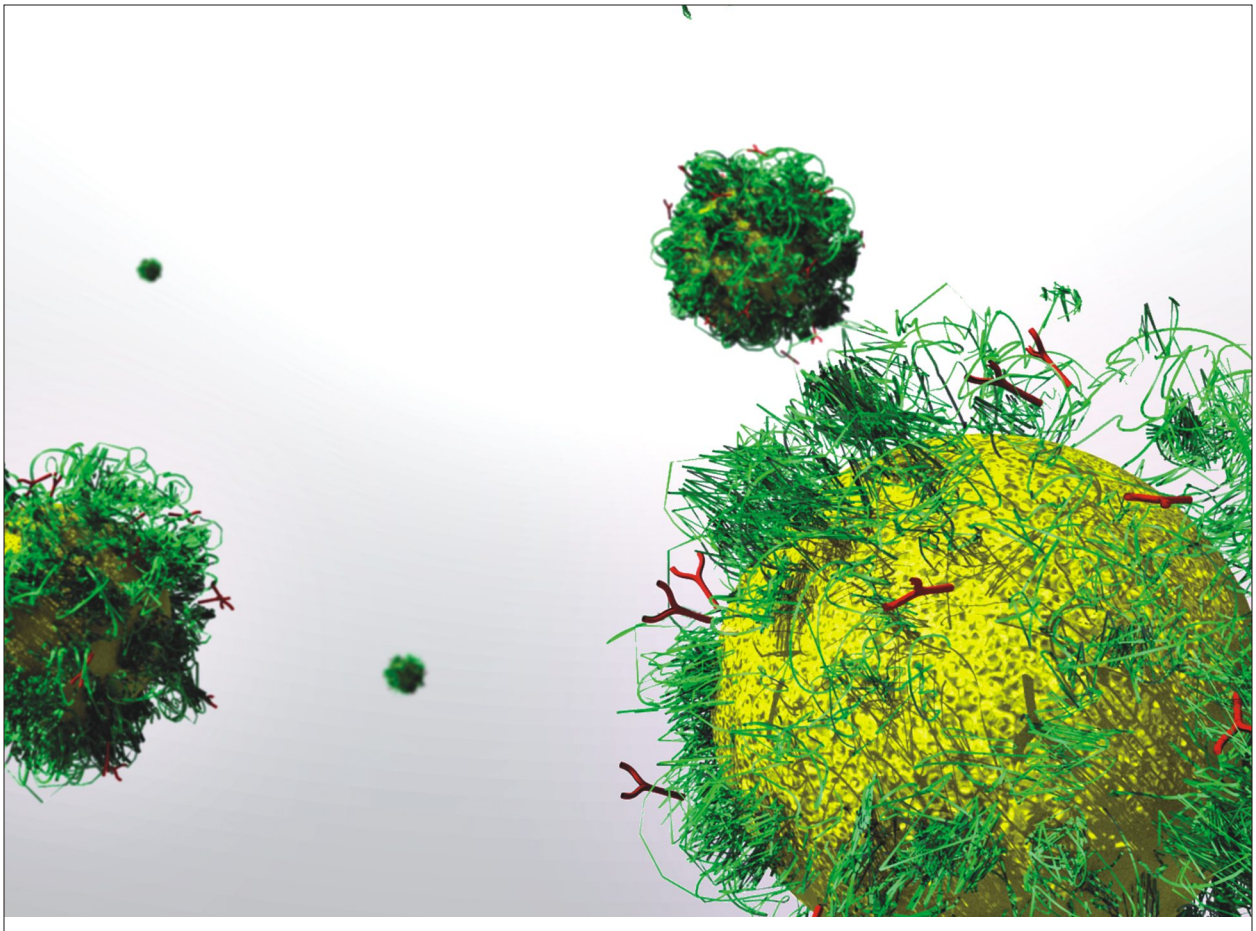

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Study on Prevalence and Antibiotic Resistance Pattern of *Klebsiella* Isolated from Clinical Samples in South East Region of Bangladesh

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ABSTRACT

The multidrug resistant *Klebsiella* is becoming an increasingly common clinical entity and has presented a global clinical problem. The present research aimed to observe the prevalence of infection and multidrug resistance pattern of *Klebsiella* in Chittagong region, Bangladesh. A total of 99 *Klebsiella* isolates associated with *Klebsiella* infection were isolated and identified according to the standard microbiological method from 502 individual patients. The most prevalent infection caused by *Klebsiella* isolates was urinary tract infection and male were more vulnerable to *Klebsiella* infection than female. Female of age 31-41 years group were found to be very much prone to *Klebsiella* associated infection whereas male has highest percentage on age group greater than 50 which was 58%. The sensitivity tests to *Klebsiella* were carried out with 5 antibiotics such as, Azythromycin, Amoxicillin, Cefexime, Nalidixic acid and Ciprofloxacin on Mueller-hinton agar by modified Kirby-bauer disc diffusion technique. Among the *Klebsiella* isolates, 92, 87, 60 and 42% were susceptible to Ciprofloxacin, Azythromycin, Cefixime and Nalidixic acid, respectively. Nearly 81% *Klebsiella* isolates exhibited resistance to amoxicillin and 58% isolate to Nalidixic acid. Azithromycin and Ciprofloxacin showed tremendous level of sensitivity to *Klebsiella* spp. The present study revealed the incidence of resistance to available antibiotics is currently relatively high, which implies a total lack of antibiotics for treatment of life-threatening infections caused by this MDR Gram-negative 'superbug'.

Key words: *Klebsiella*, nosocomial infection, antibiotic, multidrug resistance (MDR)

INTRODUCTION

Klebsiella species are considered to be major opportunistic pathogens which can cause nosocomial infections. *K. pneumoniae* is an important cause of human infections among all Klebsiella species, followed by *K. oxytoca*, *K. ozaenae* and *K. rhinoscleromatis* (Podschun and Ullmann, 1998). In recent years, *Klebsiella* species have become important and frequent pathogens in hospital-associated infections. The range of clinical diseases includes nosocomial pneumonia, urinary tract infections and bacteremia in immunocompromised humans (Chou *et al*., 2004). The emergence of multidrug resistant *Klebsiella pneumoniae* is a growing up problem around the world (Deris *et al*., 2012). Emergence and spread of antimicrobial resistance is an inevitable consequence of widespread use of antibiotics in medical practice (Finch *et al*., 2012). Carriage of *Klebsiella and* the development of multidrug-resistant bacteria have been increased due to frequent use of broad-spectrum antibiotics in hospitalized patients (Paterson and Bonomo, 2005). Antibiotic

resistance is one of the major public health concerns in this century declared by World Health Organization (WHO) (Lachmayr *et al.*, 2009). It is one of the major causes of failure in the treatment of infectious diseases that results in increased morbidity, mortality and costs of health care (Bouza and Cercenado, 2002). Furthermore, the high prevalence of bacterial infections and extensive use of antibiotics incline to higher the percentage of antimicrobial drug resistance in developing countries like Bangladesh (Okeke *et al.*, 1999; Col and O'Connor, 1987; O'Brien, 1992). In the South- East Asia (SEA) region, antimicrobial resistance (AMR) is a burgeoning and greatly ignored problem (WHO, 2011).

Surveillance of antibiotics is important to the control of resistance and *Klebsiella pneumoniae* infection. Therefore, this study was conducted to isolate, identify and determine the sensitivity pattern of *Klebsiella* from clinical specimens in Chittagong which is the second largest city of Bangladesh.

MATERIALS AND METHODS

Sample collection: A total number of 434 urine, 25 swab, 37 pus and 6 sputum, samples from August 2011-July 2012 were included in the study. All clinical samples had taken from prescribed patients who submitted their samples to Chevron Clinical Laboratory and Surgiscope Hospital. All clinical specimens were collected and processed according to standard operating procedures.

Isolation and identification of *Klebsiella*: Clinical isolates of *Klebsiella* were identified by their morphological characteristics on MacConkey agar media. The large mucoid colonies obtained from MacConkey's agar plate were processed for Gram staining. In Gram staining, all the isolates were G^- and rod shaped.

Biochemical characterization: The biochemical tests employed were IMViC (Indole, Methyl red, Voges Proskeaur, Citrate), H_2S production on TSI agar, Motility test, Oxidase test, Urease test, fermentation of Lactose and Mannitol. For biochemical tests standard procedures were used (Cruickshank, 1980).

Antibiotic susceptibility test: Identified *Klebsiella* isolates were then subjected to sensitivity analysis against five antibiotics on Mueller-hinton agar by modified Kirby-bauer disc diffusion technique. Zone of inhibition are measured according to the criteria suggested by NCCLS (2000). The antibiotics that were used included Ciprofloxacin (05 μg), Nalidixic acid (30 μg), Amoxicillin (30 μg), Azythromycin (15 μg) and Cefixime(10 μg).

Statistical analysis: Analysis was performed by using Statistical Package for Social Science (SPSS Version 16) software and Excel Office program for the statistical analysis of this study. The t-test was done as a test of significance.

RESULTS AND DISCUSSION

The comprehensive data analysis of *Klebsiella* infected patients in different age group suggested the prevalence of multidrug resistant *Klebsiella* is increasing sharply. The prevalence of *Klebsiella* infections varies in different geographical regions which create an alarming situation nowadays (Riaz *et al.*, 2012). From the screened cases, *Klebsiella* was found in 19.72%. Similar results were found earlier by Riaz *et al.* (2012), Lina *et al.* (2007) and Sarathbabu *et al.* (2012) where *Klebsiella* was mostly the etiologic agent.

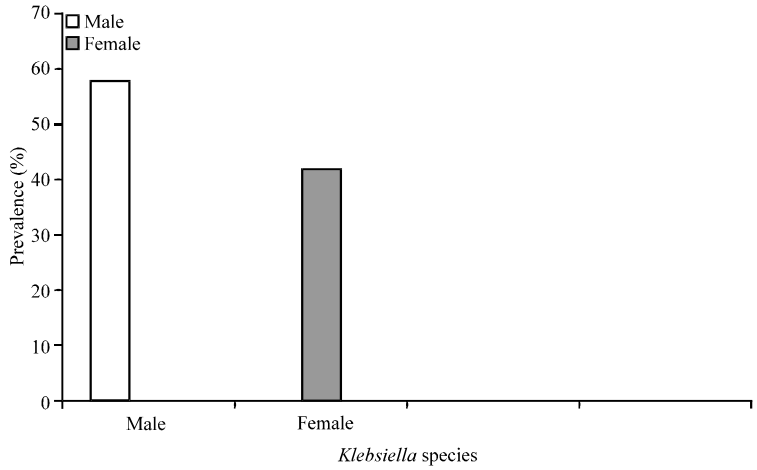

Fig. 1: Prevalence of *Klebsiella* according to gender classification

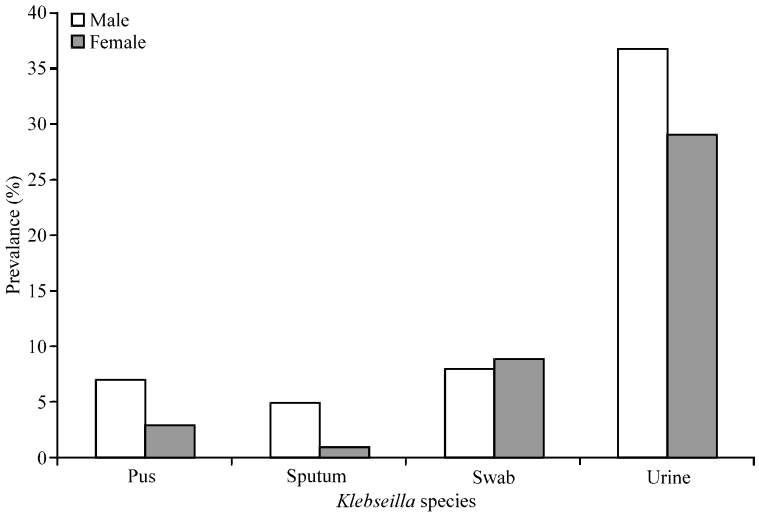

Fig. 2: Frequency of specimen sources in different genders caused by *Klebsiella* spp.

In this study, samples in the form of urine, swab, pus and sputum were collected from 502 individuals including male and female. From these 502 patients sample, 99 gave positive results for *Klebsiella* associated infection. In the case of *Klebsiella*, males (57%) had a higher incidence as compared to females (42%) considering gender classification. It depicted that male were more vulnerable to *Klebsiella* infection than female (Fig. 1). Janda and Abbott (2006) reported that males are more susceptible to infection with Klebsiella spp. than females.

In this study, data of *Klebsiella* had arranged according to clinical samples. The most prevalent infection caused by *Klebsiella* isolates was urinary tract infection followed by swab or pus. Riaz *et al*. (2012) reported that urine is a most common source of UTI infection. However, in the case of urine, pus and sputum, males were more affected with 37, 7 and 5% of *Klebsiella*, respectively. In swab samples, 9% *Klebsiella* affects females (Fig. 2).

To determine the correlation of *Klebsiella* infection with sex and age, the patient histories were analyzed. It was observed that maximum age group the percentage of female patients was higher

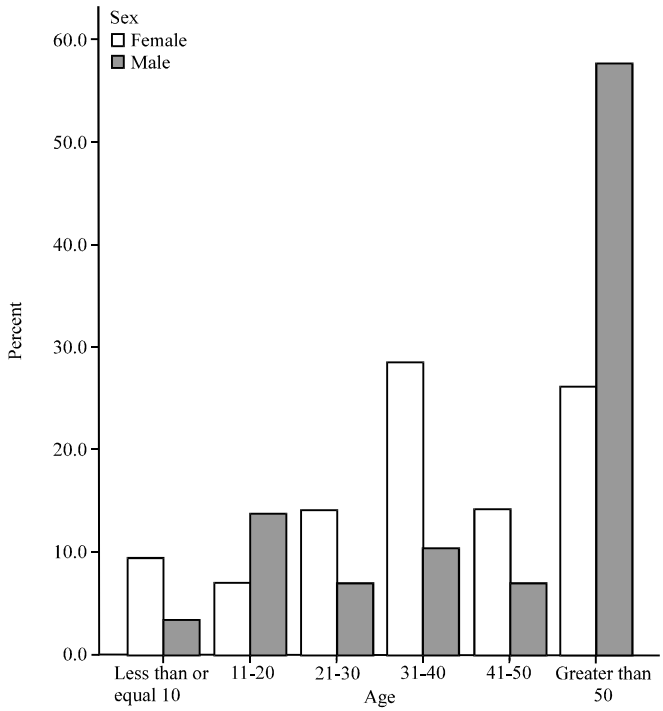

Fig. 3: Percentage of male and female patients by *Klebsiella* in different age groups in this study

than male. Male has highest percentage on age group greater than 50 which was 58%. In the age group 31-40 years, female patients had the top most percentage (approx. 29%) which was the highest figure compare to other age group (Fig. 3). In males, the age groups between 50+ and 70+ had larger frequency of beta-lactamase producing *Klebsiella* infections (Riaz *et al*., 2012). On the other hand, high frequency in females was in age groups of 70+ and 20-30+. Lina *et al*. (2007) reported men and women of elderly group were found to be very much prone to UTI. In Pakistan, both males and females in the age group of 50+ were more commonly infected by multidrug resistant bacteria (Bashir *et al*., 2008). Lindback *et al*. (2010) revealed that there are interrelations between old age and antibiotic resistance.

Month wise observation of 99 *Klebsiella* infection cases showed that, during the year, the percentage of female and male patients were fluctuating. Moreover, June-July got the peak number of patients. During June, female and male patients were 19.05 and 12.28%, respectively and in July female and male patients were 19.05 and 15.79%. Between November and December, the amounts of female patients decrease sharply. In the month of November and December male patients were 8.77 and 7.02%. This data suggested that, the onset of the diseases was high in June and July.The number of female patients were significantly high in both June and July and low in January and December (Fig. 4). Atmospheric condition of Bangladesh may play the vital factor for *Klebsiella* infection.

The problem of bacterial antibiotic resistance emerged as soon as the first antibiotics become available for clinical use (Raco and Barez, 1998). All the isolates were tested for antibacterial susceptibility. Among positive isolates, 92 and 87% were susceptible to Ciprofloxacin and Azythromycin respectively whereas only 60% was susceptible to Cefixime and 42% to Nalidixic acid. Nearly 81% *Klebsiella* isolates exhibited resistance to Amoxicillin and 58% isolate to Nalidixic acid.

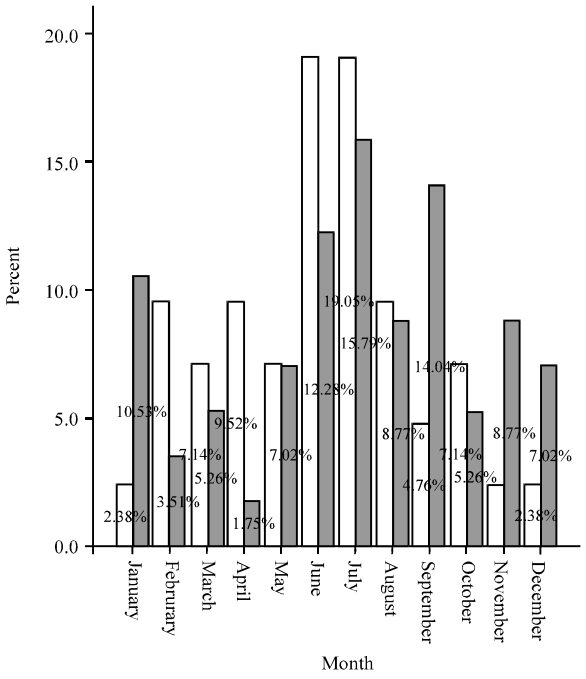

Fig. 4: Month wise observation of patients by *Klebsiella* infection in this study

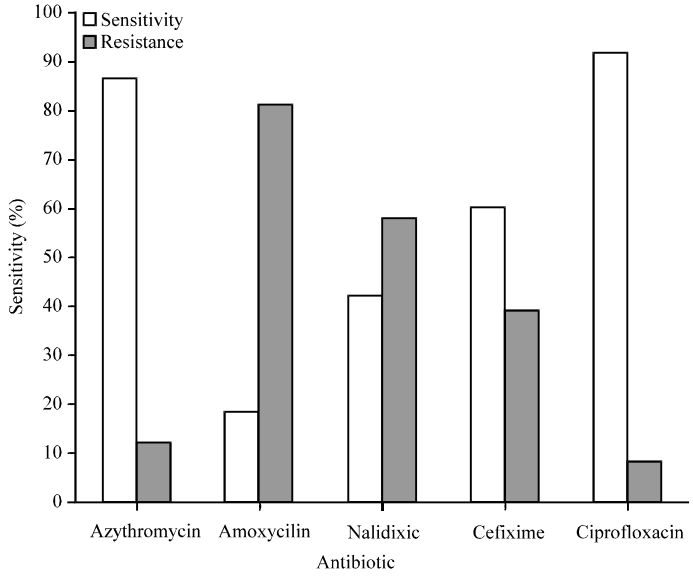

Fig. 5: Antibiotic resistance/sensitivity pattern of the *Klebsiella* spp. isolates recovered from patients

This data suggested that, Amoxicillin, Cefexime, Nalidixic acid were significantly resistant to *Klebsiella*. But Azithromycin, Ciprofloxacin showed tremendous level of sensitivity to *Klebsiella* (Fig. 5). Lina *et al*. (2007) reported, all of the *K. pneumoniae* isolates were noticed to be resistant to Ampicillin and Amoxicillin and 86% isolates were resistant to Azithromycin. Sikarwar and Batra (2011) found that *K. pneumoniae* were resistant to Ciprofloxacin and Nalidixic acid which

observed approximately 45-54% isolates. The major reason of this spread of resistance is likely due to inappropriate prescribing of antibiotics, incorrect dose or duration of use and antibiotic use without prescription.

CONCLUSION

The emergence of Multidrug-resistant (MDR) *Klebsiella* is inevitable and is a major public health threat worldwide. In Chittagong, numerous hospitals have experienced outbreaks of infections caused by MDR *Klebsiella*. This study suggested that, 92, 87, 60 and 42% *Klebsiella* isolates were susceptible to Ciprofloxacin, Azythromycin, Cefixime and Nalidixic acid respectively. Nearly 81% *Klebsiella* isolates exhibited resistance to Amoxicillin and 58% isolate to Nalidixic acid. Azithromycin and Ciprofloxacin showed tremendous level of sensitivity to *Klebsiella* spp. Therefore, the present study provides an idea about the prevalence of *Klebsiella* as superbug in Chittagong city, which needs special attention for improving the health care systems of Bangladesh.

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