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original research article

Prevalence, antibiotic susceptibility profiles and ESBL production in *Klebsiella pneumoniae* and *Klebsiella oxytoca* among hospitalized patients

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Abstract

Background and Purpose: Klebsiella pneumoniae *and* Klebsiella oxytoca *are the two most common pathogens causing nosocomial infections in humans and are of great concern for developing multidrug resistance. In the present study,* K. pneumoniae *and* K. oxytoca *from clinical samples were evaluated for their antibiotic sensitivity patterns against commonly used antibiotics and production of extended-spectrum beta-lactamase (ESBL).*

Materials and Methods: *The isolates were obtained from tracheal swabs, sputum, wound swabs, pus, blood and urine samples of hospitalized patients.* Klebsiella pneumoniae *and* Klebsiella oxytoca *were identified by cultural and biochemical methods. Antibiotic sensitivity test was performed by modified Kirby-Bauer disc diffusion technique. ESBL production in* Klebsiella *spp. was confirmed by double disc synergy test.*

Results and Conclusion: *Out of 500 clinical isolates, 120 were found positive for* Klebsiella *among which 108 were* K. pneumoniae *and 12 were* K. oxytoca *based on indole test. Prevalence rate of* Klebsiella *was found more prominent in males aged over 50 years, mostly in urine samples. Overall resistance pattern of* Klebsiella *isolates to Ampicillin, Amoxicillin, Ceftriaxone, Ciprofloxacin, Co-trimoxazole, Gentamicin, Nalidixic acid, Tetracycline was 100%, 90%, 45%, 40%, 45%, 25%, 50%, 35% respectively. Multidrug resistance was found more common in* K. pneumoniae *(56%) than in* K. oxytoca *(50%). Prevalence rate of ESBL producing* Klebsiella *was found 45% among which* K. pneumoniae *(50%) were found more prominent than* K. oxytoca *(25%). All the ESBL producing* Klebsiella *isolates were found to be multidrug resistant, showing 100% resistance to Ampicillin, Amoxicillin, Ceftriaxone and Ciprofloxacin.*

INTRODUCTION

Klebsiella have been considered as one of the major opportunistic pathogens that cause a range of clinical diseases including nosocomial pneumonia, urinary tract infections and bacteremia in immunocompromised humans *(8)*. Among the five gram-negative pathogens for hospital-acquired infections, *Klebsiella* is the most commonly encountered pathogen *(14)*. The leading *Klebsiella* species giving rise to infections in humans are *Klebsiella pneumoniae* and *Klebsiella oxytoca (13)*. *K. pneumoniae* accounts for 75% to 86% of all *Klebsiella* species reported while *Klebsiella oxytoca* accounts for 13% to 25% of isolates *(12, 38)*.

Emergence of antimicrobial resistance among *Klebsiella* species is becoming a global public health concern. High rate of antibiotic resistance is an inevitable consequence of widespread use of antibiotics in medical practice in developing countries *(28, 30)*. In addition, resistance to broad spectrum beta-lactam antibiotics via extended-spectrum beta-lactamase (ESBL) production is an increasing problem worldwide *(24)*. *Klebsiella* and *E. coli* were reported to be the most common pathogenic microorganisms to develop resistance to broad spectrum beta-lactam antibiotics via extended-spectrum beta-lactamase (ESBL) *(2)*. Several risk factors for ESBL-producing *Klebsiella* spp. infections have been described for the most frequent antimicrobial exposure, especially to third-generation cephalosporins *(18, 25)* which resulted in increased morbidity, mortality and costs of health care. Failure in the treatment of infectious diseases especially caused by *Klebsiella*, therefore, need to be under controlled monitoring in developing countries like Bangladesh to avoid widespread distribution of multidrug resistant *Klebsiella* spp.

Therefore, the present study aims to evaluate the prevalence of *K. pneumonia* and *K. oxytoca* among clinical patients and to analyze ESBL production and antimicrobial susceptibility pattern against most commonly prescribed antibiotics in order to treat patients with diseases caused by *K. pneumonia* and *K. oxytoca*.

MATERIALS AND METHODS

Sample collection and processing

A total of 500 clinical samples including blood, urine, pus and sputum were obtained from Popular Diagnostic Centre and Medinova Diagnostic Centre of Sylhet branch, Bangladesh from November 2013 to October 2014. The patients were of different ages and also of both sexes. The collected samples were then collected and processed according to standard operating conditions.

Isolation and identification of Klebsiella

Klebsiella were isolated and identified by culturing the clinical samples onto MacConkey agar plate where large mucoid colonies were selected. The selected colonies were further confirmed by culturing on Eosine Methylene Blue (EMB) agar plate. Then the presumptively identified *Klebsiella* colonies were checked for their Gram staining reaction.

Biochemical characterization

Biochemical tests employed were IMViC (Indole, Methyl red, Voges Proskeaur, Citrate), fermentation of sugars, oxidase and catalase test. For biochemical tests standard procedures were used *(7)*.

Antibiotic susceptibility test

Susceptibility of bacterial isolates to different antibacterial agents was determined on Mueller-Hinton agar by modified Kirby-Bauer disc diffusion technique. The 0.5 McFarland standard isolates were inoculated with sterile cotton swab onto Mueller Hinton agar to make a lawn of bacterial growth. The plates were incubated overnight at 37°C and zone of inhibition were measured according to the criteria suggested by NCCLS, 2000 *(27)*. Commercially available antimicrobial discs (Hi-Media Laboratories Pvt. Limited, India) that were used included Ampicillin (25 µg), Amoxicillin-clavulanic acid (30 µg), Ciprofloxacin (05 µg), Co-trimoxazole (25 µg), Ceftriaxone (30 µg), Gentamicin (10 µg), Tetracycline (30 µg) and Nalidixic acid (30 µg).

Detection of ESBLs

Isolates that exhibited reduced susceptibility to ceftriaxone (30 µg) were considered as potential producers of ESBL *(27)*. ESBL production in *Klebsiella* spp. was confirmed by double disc synergy test as described by Jarlier, 1988 *(17)*. An inoculum of 0.5 Mcfarland standard was inoculated with sterile cotton swab onto Mueller Hinton agar to make a lawn of bacterial growth. 30 µg disc of Ceftriaxone (30 µg) was placed on the agar plate at a distance of 20 mm centre to centre from a Amoxicillin-clavulanic acid (20/10 µg) disc prior to incubation. The plate was incubated overnight at 37°C. Enhancement of the zone of inhibition of Ceftrioxone disc toward Amoxicillin-clavulanic acid confirmed the presence of extended-spectrum β-lactamases.

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

Prevalence of Klebsiella (K. pneumonae and K. oxytoca)

Out of 500 samples from clinical patients, *Klebsiella* spp. were reported in 120 cases through standard cultural and biochemical tests. Out of these 120 *Klebsiella spp.* 108 were *K. pneumoniae* and 12 were found *K. oxytoca* based on indole test. The prevalence rate of *Klebsiella* was about 24% among which *K. pneumoniae* accounted in 21.6% cases and *K. oxytoca* in 2.4% cases (Fig. 1).

Highest prevalence was observed in case of urine sample (66%), followed by swab in 17% cases, pus in 10% cases and sputum in 7% cases (Fig. 2). Males were more affected (57%) with *Klebsiella* spp. than females (43%) (Fig. 3).

Persons older than 50 years irrespective of gender were more affected with *Klebsiella* spp., 58% in male and 36%

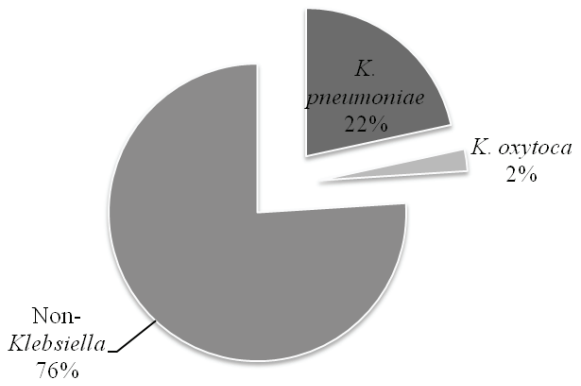

Fig. 1. *Prevalence rate of K. pneumoniae and K. oxytoca among clinical samples.*

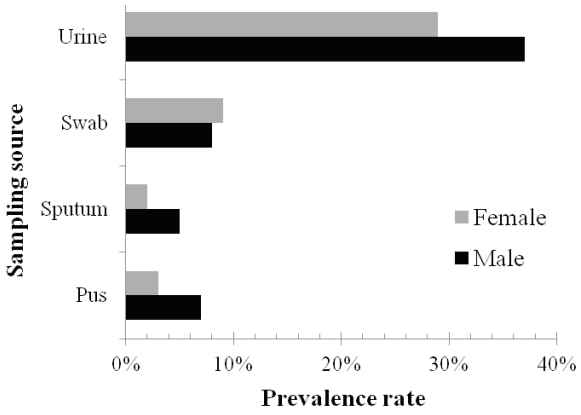

Fig. 2. *Occurrences of Klebsiella spp. in different clinical samples.*

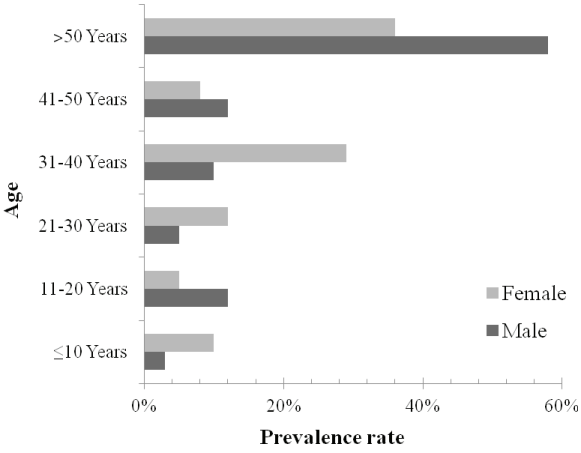

Fig. 3. *Occurrences of Klebsiella spp. among different age groups.*

in case of female (Fig. 3). The occurrence of infection due to *Klebsiella* spp. was found almost equivalent throughout the 12 months with some inclination in the months of June (12% for male and 19% for female) and July (16% for male and 19% for female) (Fig. 4).

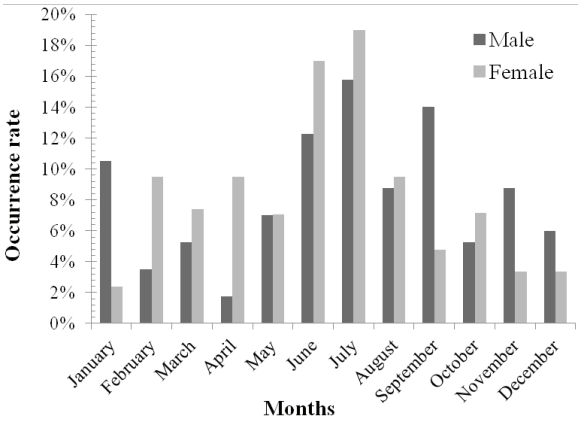

Fig. 4. *Monthly occurrence rate of Klebsiella spp.*

Antibitic sensitivity pattern of Klebsiella

The resistance pattern of *Klebsiella* isolates to Ampicillin, Amoxicillin, Ceftriaxone, Ciprofloxacin, Co-trimoxazole, Gentamicin, Nalidixic acid, Tetracycline was 100%, 90%, 45%, 40%, 45%, 25%, 50%, 35% respectively (Fig. 5). Highest sensitivity was observed against Gentamicin (75%) followed by Tetracycline (65%). Multidrug resistance (MDR) was observed in 55% of *Klebsiella* isolates where 12 isolates out of 120 were found resistant against all the antibiotics tested.

Antibiogram of *K. pneumoniae* revealed resistance to Ampicillin, Amoxicillin, Ceftriaxone, Ciprofloxacin, Cotrimoxazole, Gentamicin, Nalidixic acid, Tetracycline by 100%, 94%, 50%, 37.5%, 44%, 31%, 44% and 31% respectively (Fig. 6). Antibiotics that were found most potent against *K. pneumoniae* was Gentamicin and Tetracycline (69% in both cases) followed by Ciprofloxacin (62.5%). Multidrug resistance was observed among 56% *K. pneumoniae* isolates.

Our data showed that *K. oxytoca* was 100% resistant towards Ampicillin which was similar to *K. pneuminiae*. However, unlike *K. pneumoniae*, *K. oxytoca* revealed resistance to Amoxicillin, Ceftriaxone, Ciprofloxacin, Co-

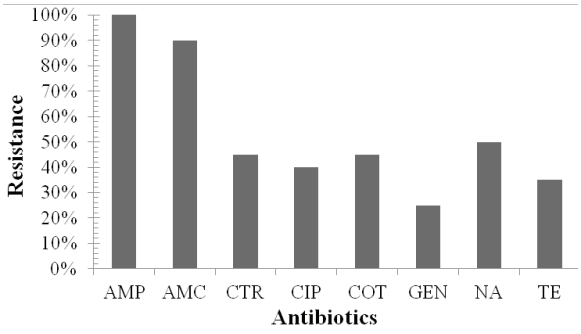

Fig. 5. *Antibiotic resistance pattern of Klebsiella isolates.*

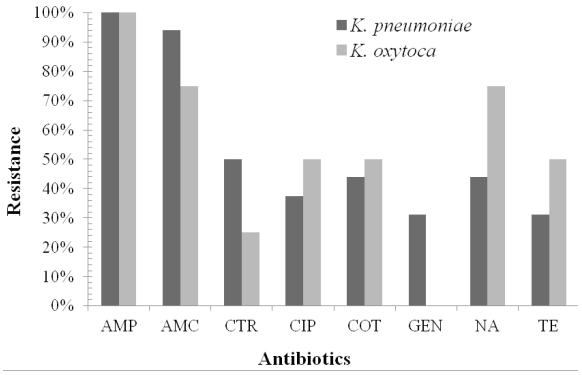

Fig. 6. *Comparative antibiotic resistance pattern of K. pneumoniae and K. oxytoca.*

trimoxazole, Gentamicin, Nalidixic acid, Tetracycline by 75%, 25%, 50%, 50%, 0%, 75% and 50% respectively (Fig. 6). Gentamicin was found to be the most potential drug followed by Ceftrioxone. Multidrug resistance was observed among 50% *K. oxytoca* isolates.

Potential ESBL among Klebsiella spp.

Primarily 65% of the *Klebsiella* isolates were identified as potential ESBL producer through the critical zone diameter concept, among which 69% were *K. pneumoniae* and 50% were *K. oxytoca*. However, the double disc synergy test revealed that 45% *Klebsiella* isolates possess ESBL property among which *K. pneumoniae* were 50% and *K. oxytoca* were 25%.

DISCUSSION

The high prevalence rate of *Klebsiella* among clinical patients has become a global concerns in recent years. In the present study, 24% prevalence rate of *Klebsiella* was observed among clinical patients from north eastern part of Bangladesh which is alarming since less prevalence rate (19.72%) was observed previously in south eastern part of Bangladesh by Akter *et al.*, 2014 *(3)*. This difference in prevalence rate may be acceptable because the prevalence of *Klebsiella* infections varies in different geographical locations *(33)*. The two clinically relevant species, *K. pneumoniae* and *K. oxytoca* was differentiated by the ability to produce indole from tryptophan *(21)*. Majority of the isolates in the present study proved to be *K. pneumoniae* (90%) which is in good agreement with previous studies *(12, 38)*. For both organisms, however, prevalence was higher in males than in the female samples. Female in the age group of 31-40 years and greater than 50 years were found to be very much prone to *Klebsiella* associated infection whereas male has highest incidence of infection on age group greater than 50 years. Similar results have been previously reported by several investigators in Bangladesh who reported that men and women of elderly group were found to be very much prone to *Klebsiella* infection *(3, 20)*. In Pakistan, males of older age and females of reproductive age were more prone to *Klebsiella* infections particularly by ESBL producers *(33)*. Most of the *Klebsiella* were recovered from urine samples irrespective of age and gender, followed by swab, sputum and pus. Akter *et al.*, 2014 *(3)* and Riaz *et al.*, 2012 *(33)* also reported that urine is the principal source of *Klebsiella*.

Our data suggest that rates of *Klebsiella* incidence reach to the peak in the month of June and July, which are the two most humid and warmest months in Bangladesh. Such findings have been also observed by Anderson *et al.*, 2008 *(4)* who demonstrated that environmental pressure during the warmest months may lead to an increase in *Klebsiella* infection. Characteristics of *Klebsiella* might have some role on such seasonal variation. *Klebsiella* are the most heat tolerant among all enteric pathogens *(29)* having maximal specific growth rate at 37°C *(9)*. In addition, they are believed to survive at higher humidity *(36)*.

The effectiveness of currently available antibiotics is decreasing due to the increasing number of resistant strains causing infections *(26)*. Available therapeutic options for antibiotic-resistant organisms are severely limited, as these organisms frequently display a multidrug-resistant (MDR) phenotype *(19, 23, 31)*. In the present study, *K. pneumoniae* and *K. oxytoca* demonstrated varying degree of sensitivity pattern to different antibiotics. Most of the *Klebsiella* isolates showed resistance to the first line antibiotics that are commonly prescribed by the physicians. Our data showed that all the *K. pneumoniae* isolates were resistant to Ampicillin. Moderate level sensitivity were observed against Ceftriaxone, Ciprofloxacin, Co-trimoxazole, Gentamicin, Nalidixic acid and Tetracycline among which Gentamicin and Tetracycline showed good promise. Gentamicin like aminoglycosides have a good record against clinically important Gram-negative bacilli *(10)*. Recently in India and Pakistan, about 16.70% and 17.39% *Klebsiella* isolates were found sensitive against Gentamicin *(32, 37)*. The difference among our data and the previously found lower sensitivity against Gentamicin may be due to selective pressure in different regions *(22)*.

Emergence of multidrug resistant *Klebsiella* has become a major public health concern worldwide and has been associated with outbreaks of hospitalized infections in developing countries like Bangladesh due to the indiscriminate use of antibiotics *(3)*. In the present study, multidrug resistance was found more common in *K. pneumoniae* (56%) than in *K. oxytoca* (50%). Various degrees of multidrug resistance among *Klebsiella* have been reported in different parts of the world. In Bangladesh, about 87% multidrug resistant *Klebsiella* were recorded *(20)* whereas in India and Pakistan, 54% and 71.73% *Klebsiella* isolates were reported to harbor multidrug resistance *(35, 37)*. Chikwendu *et al.*, 2010 in Nigeria reported 75.8% multidrug resistant *Klebsiella* isolates *(6)*. Most recently, Gundogan and Avci, 2013, reported that 31.7% *Klebsiella* isolates possess multidrug resistance *(11)*.

ESBL producing *Klebsiella* pose great challenges for efficient treatment of infections which requires new antibacterial compounds every now and then. Our study focused on the present status of ESBL producing *Klebsiella*. Interestingly all the ESBL producing *Klebsiella* isolates were found to be multidrug resistant, showing 100% resistance to Ampicillin, Amoxicillin, Ceftriaxone and Ciprofloxacin indicating relationship between multidrug resistance and ESBL possession. However, 12.5% non-ESBL *K. pneumoniae* possess multidrug resistance property while 33% of the non-ESBL *K. oxytoca* harbour multidrug resistance.

The prevalence rate of ESBL producing *Klebsiella* is very high (45%) in our study as compared to previous studies such as 40% in France *(5)* and 17% in Pakistan *(33)*. However, the present result for the occurrences of ESBL among *Klebsiella* isolates is significantly better than previous studies by Sharma *et al.*, 2013 *(34)* and Jain and Mondal, 2007 *(15)* who found much higher rate (67.04% and 58%, respectively). ESBL producing *K. pneumoniae* (50%) were found more prominent than *K. oxytoca* (25%). In case of ESBL producing *K. oxytoca*, our result is in good agreement with the studies by Gundogan and Avci, 2013, who found that 26% *K. oxytoca* were ESBL producer *(11)*. However, in case of ESBL producing *K. pneumoniae*, the rate is much higher than found by Gundogan and Avci, 2013 (38.5%) *(11)*. Such high rate of ESBL among *K. pneumoniae* might be due to inappropriate use of antibiotics and self-medication against *K. pneumoniae* infection. However, our results are significantly better for ESBL producing *K. pneumoniae* than found by Afifi (2013) in Egypt (56.25%), Jain *et al.* (2003) in India (86.6%) and Ullah *et al.* (2009) in Pakistan (58.5%) *(1, 16, 37)*.

The high frequencies with which antibiotics are used empirically to treat various diseases in Bangladesh suggest that there might be high rate of failure associated with eradication of microbial infections. Our results suggest that there is high antibiotic resistance towards commonly prescribed antibiotics and ESBLs rates among clinical *Klebsiella* isolates in the specific region of the country. Further studies need to be carried out from other parts of Bangladesh considering multidrug resistance and ESBL production rate among clinical and non clinical *Klebsiella* isolates. Continuous monitoring of ESBL, strict antibiotic policy along with conventional antibiogram will have a great impact in reducing bacterial resistance towards antibiotics and development of proper treatment options against *Klebsiella* infections.

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