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Antibiotic susceptibility pattern of Klebsiella isolates at University Teaching Hospital, Ilorin

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Abstract

Klebsiella, a major component of the normal intestinal flora is an important member of the family Enterobacteriaceae. They account for 80 percent of clinical significant isolates. The aim of this study is to isolate Klebsiella species and determine their antibiotic susceptibility pattern. Klebsiella species were isolated from various clinical samples brought to the microbiology laboratory. All routinely identified Klebsiella isolates were characterised by sub culturing onto MacConkey agar. The sensitivity rate of the isolates to some selected, popularly used and readily available antibiotics was determined. Beta lactamase production of the isolates was determined by the double disc synergy test(DDST). About One hundred and sixty-four isolates of Klebsiella were examined. Two species of Klebsiella were identified, and they are Klebsiella pneumoniae 88.0% and Klebsiella oxytoca 12.0%. Thirty-two of the isolates were found to contain ESBL. The most active antibiotic was Piperacillin 100%,Imipenem 98.2% Sulbactam,74.9% and Azithromycin 73.2%. The isolates that expressed extended spectrum beta-lactamase were susceptible to Imipenem and Piperacillin, moderate sensitivity to Ofloxacin 69.9% and Ciprofloxacin 56.3%. The isolates were resistant toAugmentin72.8%,Cefuroxime 72.5%, Ceftriaxone60.0%, The study recommends the effective use of the laboratory for determination of the sensitivity pattern of clinical isolates rather than relying on empirical use of antibiotics, because the sensitivity pattern of these clinical isolates keep changing.

Keywords: Klebsiella, Isolates, Sensitivity Pattern, ESBL.

Introduction

Klebsiella is a genus of the family *Enterobacteriaceae* and members of this genus are defined as Gram negative, non- motile, capsulated aerobic to facultative anaerobic bacilli; which are catalase positive and oxidase negative[1]. *Enterobacteriaceae* are major component of the normal intestinal flora but are relatively common in other body sites[2]. They are major cause of nosocomial infections and may account for 80% of clinically significant isolates of Gram negative bacilli in clinical microbiology laboratories and 5% of all clinically significant isolates[3,4,5].

Klebsiella are isolated in many types of human infections, such as abscesses, pneumonia, meningitis, septicaemia, intestinal and urinary tract infections[6,7,8]. Hospitalized patients often become colonized with *Klebsiella* species and among the *Enterobacteriaceae, they*are major cause of hospital acquired infections[5].Nosocomial *Klebsiellapneumoniae* is associated with a high mortality in both neonates and adults and antimicrobial treatment of such infections has been complicated by the emergence of multidrug resistant strains[9] *Enterobacteriaceae* are known for their production of enzymes known as beta lactamase which degrade the beta lactam ring of the beta lactam antibiotic group such as the Penicillin's and the Cephalosporins[10].

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There is one group of beta lactamases that is essentially found in certain species of Gram negative bacilli usually in *Klebsiella* and Escherichia coli; and these enzymes are termed extended spectrum beta lactamase[11,12]. These enzymes confer upon the bacterial the additional ability to hydrolyse the beta lactam ring of Cephalosporin such as Cefotaxime, Ceftazidime and Azetreonam. Several outbreaks of infection with ESBL-producing*Klebsiellaspp* have been reported from South Africa[13], Ghana[14], and in many centres in Nigeria[17]. The aim of this study is to determine the antibiotic sensitivity pattern of *Klebsiella* isolates from clinical samples.

Materials and Method

This study was conducted at the microbiology laboratory of the University of Ilorin Teaching Hospital between June and December 2015. A total of 164 isolates were examined. *Klebsiella*isolates from swabs, urine, blood culture, CSF, Semen, and Sputum constituted the sample size. All routinely identified *Klebsiella* species were characterized by sub culturing at 37^0c. The organisms that conform to the genus were further tested biochemically to differentiate them in to species. The tests were glucose fermentation for acid and gas production, Dulcitol and Glucose fermentation at 5^0c. The other tests include Methyl Red (MR) and Voges ProsKauer (VP).

Extended spectrum beta lactamase was detected in isolates that were found to be resistant to Ceftazidime, Ceftriaxone and Cefotaxime, using double disc synergy test (DDST).Plates of Mueller-Hinton agar were prepared with Ceftazidime and Cefotaxime in a concentration of 30 µg were placed 15mm center to cneter from Amoxicillin-clavulanic acid disc (20 and 10 µg). The inoculated media were incubated for 18-24 hours at 37^0c. Enhanced zone of inhibition between any of the beta lactam discs and the disc at the center were recorded. Antibiotic susceptibility test was performed on all the isolates using the disc diffusion method (CLS1 2010)[18]. The zone of inhibition of the antibiotics were measured and interpreted as per Clinical laboratory standard institute recommendations[18]. Eleven different antibiotic discs commonly prescribed by the clinicians at the University of Ilorin Teaching Hospital were used and these are Gentamicin (10µg), Ciprofloxacin (5µg), Imipenem (10µg), Augmentin (30µg), Azithromycin (15µg), Ofloxacin (5µg), Cefuroxime (30µg), Ceftriaxone (30µg), Ceftazidime (30µg), Sulbactam (10µg), and Piperacillin (100µg), Escherichia coli ATCC (25922) strain were used as control culture.

Results

Table 1: Biochemical characterization of *Klebsiella* species

Klebsiella Spp	Lac	Duc	Gas in GlucGlu/5^0c		MR	VP
Pneumoniae	+	+	+	+	+	-
Oxytoca	+	+	+	-	-	+
Ozaenae	+	-	+	-	-	-
Planticola	+	+	+	+	-	+
Rhinoscleromatis	-	-	-	-	+	-

Table 2: Sensitivity and Resistance pattern of the isolates.

Type of Antibiotic	**No tested**	**No. sensitive(%)**	**No. resistance(%)**
Azithromycin	**92**	**67 (72.2)**	**25 (27.8)**
Sulbactam	**46**	**34 (74.9)**	**12 (26.1)**
Augmentin	**103**	**28 (27.2)**	**75 (72.8)**
Imipenem	**56**	**55 (98.2)**	**1 (1.8)**
Piperacilin	**26**	**26 (100.0)**	**0 (0.0)**
Gentamicin	**117**	**61 (52.1)**	**56 (47.9)**
Cefuroxime	**80**	**22 (27.5)**	**58 (72.5)**
Ofloxacin	**73**	**51 (69.9)**	**22(30.1)**
Rocephin	**130**	**52 (40.0)**	**78 (60.0**
Ceftazidime	**109**	**65 (60.0)**	**44 (40.0)**
Ciprofloxacin	**119**	**67 (56.3)**	**52 (43.7**

Table 3: Pattern of infections caused by the isolates.

Diagnosis	**No**	**(%)**
Diabetes Mellitus	**4**	**2.4**
Osteomyelitis	**3**	**1.8**
Meningitis	**2**	**1.2**
Cerebro Vascular Accident	**2**	**1.2**
Chronic Renal Disease	**1**	**0.6**
Abscesses	**3**	**0.6**
Burns	**2**	**1.2**
Cathether	**2**	**1.2**
Cellulitis	**1**	**0.6**
Ear discharge	**22**	**13.4**
Septicaemia	**77**	**47.0**
Wound infections	**38**	**23.2**
Urinary Tract Infection	**9**	**5.5**
Total	**166**	**100**

Table 4: Gender distribution of the isolates.

Sex	Frequency	Percent
F	**76**	**45.8**
M	**90**	**54.2**
TOTAL	**166**	**100**

Discussion

Two species of *Klebsiella* were identified in this study and these are*K. pneumoniae* and *K. oxytoca*. *K. pneumoniae* was the predominant species and this accounts for 88.0% of the total isolates. This is similar to the findings of a study done in Lagos, Nigeria; where over 90% of the clinical isolates of *Klebsiella* were K. pneumoniae[19]; but at variance with another study from same location where four species of *K.pneumoniae* were isolated[20]. Usually the most frequently isolated strains of *Klebsiella*is*K.pneumoniae* which is a recognized pathogen. It accounts for large numbers of hospital and community acquired infections involving the urinary tract, blood and lungs[21].

Antimicrobial resistance among Gram-negative bacilli is a major problem in hospital acquired infections with increasing morbidity and mortality more especially when infections are caused by drug resistant organisms[22]. In this study, the most effective antibiotic is Piperacillin with a sensitivity pattern of 100% and this agrees with the finding of a study in India where Piperacillin was equally found to be 100% sensitive to all the clinical isolates[23]. This finding is at variance with the report of a studywhere*K.pneumoniae* isolates were resistance to *Piperacillin*. This antibiotic is not commonly prescribed by physicians in the location of the present study. In addition, Piperacillin is an injectable and it is usually given in combination with another drug called Tozobactam. Injectable generally are rarely abused or misused by patients except for the over use by physician. Piperacillin-Tozobactam is a very useful combination against Klebsiella strains with extended spectrum beta lactamase production. Nevertheless, some ESBL producers have been found to be resistant to Piperacillin[24].

Another remarkable antibiotic with high sensitivity is Imipenem, with a sensitivity of about 98.2% to the isolates of *Klebsiella* species. This finding however agrees with the report of Namaratha and others who recorded 100% sensitivity of *Klebsiella* to Imipenem [25,26]. This report also correlates with the findings of Innocent in Uyo. High sensitivity of Imipenem in this environment could be due to the high cost of the drug. Imipenem is expensive and it is only in injectable. These prevents its adulteration and abuse hence its high sensitivity to bacterial infection.

Sulbactam however in the study is found to be effective against the *Klebsiella* isolates. The sensitivity in this present study is 74.9%. The antibiotic is mostly used in the hospitals and the chance of been abused is less. It is commonly used in the Paediatric populations and rarely prescribed for adults in the area of the study as compared to the oral preparations.

Ciprofloxacin and Ofloxacin are the two Quinolones tested in this study. The isolates are still moderately susceptible to Ofloxacin with a sensitivity pattern of 69.9% and the sensitivity of Ciprofloxacin is on the downward trend with a sensitivity of 56.3%. This finding is at variance with result of Boma and friends who demonstrated that Quinolones was the most potent antimicrobial against Gram negatives, more especially *Klebsiella* isolates. In their study both Ofloxacin and Ciprofloxacin were found to be 90% effective against *Klebsiella* species[27]. The Quinolones nowadays are commonly prescribed and are readily available over the counter. Ciprofloxacin are more often prescribed more than Ofloxacin and this could be responsible for the decreasing sensitivity because of over use.

The isolates were found to be resistant to the Cephalosporins in use except Ceftazidime which shows a sensitivity of 65%. However,the isolates were 78.0% and 58.0% resistant of Ceftriaxone and Cefuroxime respectively. The antibiogram pattern of the isolates in this study revealed a decrease in sensitivity to the commonly used antibiotics. The resistance of the isolates to Cephalosporins is an indication of ESBL production. Some isolates were found to be multidrug resistant and were found positive for ESBL production. This supports the findings of other scientists [28,29] where *Klebsiella* was found to be resistant to Cefuroxime and Ceftriaxone. The emergence of resistance of *Klebsiella* to the Cephalosporin have been widely reported[30,31,32].

The preponderance of the isolates was from males. *Klebsiella*, a pyogenic organism was seen to cause a variety of infections in Ilorin. Most the isolates were from blood 47.0%, wound 23.2% and ear discharges

13.4%. The ability of *Klebsiella* to evade host immune system and the production of enzymes such as Extended Spectrum Beta Lactamase can be responsible for the high rate of septicaemia in this study.

The bulk of the isolates were seen in the age group 1-10 years, this is contrary to expectation. Persons with alcoholism are usually the main population at risk and they contribute 66% of people usually affected by this infectious agent[39,33].

Conclusion

This study has shown that *Klebsiella* species are isolated in many types of infection in our environment and *Klebsiella pneumoniae* is the predominant species. Some of the isolates are ESBL producers, although the prevalent is not high. The use of some first line antibiotics such as Cefuroxime, Ceftriaxone and Augmentin are inappropriate in the treatment of infection caused by these isolates. Piperacillin and Imipenem were the most sensitive drugs and could be administered empirically. Though expensive but are cost effective. However, the issue of cost could be addressed by effective implication of the National Health Insurance Scheme. The studysuggests an effective antibiotic control policy to prevent further escalation of ESBL producers in this environment. The clinicians should rely on the laboratory sensitivity pattern of isolates rather than the use of empirical antibiotics in the management of infection caused by *Klebsiella* species.

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