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Original Research Article

Evaluation of Two Phenotypic Methods for the Confirmatory Testing of Extended Spectrum Beta Lactamases Producing Strains of *Klebsiella Pneumoniae*

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ABSTRACT

Background: The resistance to antimicrobials has become a serious global health concern with negative consequences on treatment strategies and increasing health-care costs. The extended spectrum beta lactamases producing bacteria stands outs as bacteria of great concern among Gram negative bacilli. Lack of capacity to effectively diagnose these organisms in developing countries result in sub-optimal treatment. We compared two phenotypic methods for the confirmatory testing of ESBL in *Klebsiella pneumoniae* to identify an easy and efficient method for their laboratory diagnosis

Materials and Methods: We screened all patients admitted into various units of University of Maiduguri Teaching Hospital between from the 01/01/2014 to 31/06/2014 to isolate *Klebsiella pneumoniae*.

All confirmed isolates were screened for ESBL enzyme using CLSI breakpoints. Suspected ESBLs producers were subjected to confirmation using two phenotypic methods. The double disk synergy method (with ceftazidime; 30 µg, cefotaxime;30 µg, and amoxicillin;20 µg, plus clavulanate;10µg,:[augmentin;30 µg].) and Etest method for MIC determination (using cefotaxime and cefotaxime + amoxicillin-clavulanic acid).

Multiplex polymerase chain reaction (PCR) method was considered as a gold standard for confirmation. We compared the two methods with the gold standard to determine their sensitivity, specificity and predictive value positive.

Results: We detected 178 isolates of *Klebsiella pneumoniae* among of hospitalized patients. The DDST method revealed 59 out of the 178 isolates resistant with a sensitivity of 100%, specificity of 97%, positive predictive value of 93% and negative predictive value of 100%.

Using the Etest MIC, 56 resistant isolates were identified with a sensitivity of 100%, specificity of 99%, positive predictive value of 98% and negative predictive value of 100%.

Only 55resistant organisms were found based on the multiplex Polymerase chain reaction method.
Conclusion: The two methods of DDST and Etest MIC used shows high validity with Etest MIC MIC having a relatively higher specificity. However, in view of cost, the DDST is recommended for use in our clinical laboratories.
Key words: Resistance, Cefotaxime, Cephalosporins, Methods, Klebsiella pneumoniae, Double disk synergy.

INTRODUCTION

The resistance to antimicrobials has become a serious global health concern with negative consequences on treatment strategies and increasing health-care costs. [(1)] The extended spectrum beta lactamases producing bacteria stands outs as bacteria of great concern among Gram negative bacilli. Lack of capacity to effectively diagnose these organisms in developing countries result in sub-optimal treatment. [(2, 3)]

Extended spectrum beta lactamases are plasmid mediated enzymes that are capable of conferring bacterial resistance to the penicillin's, first, second and third generation cephalosporin's and aztreonam. They do this by hydrolysis of these antibiotics but they are inhibited in vitro by beta lactamase inhibitors such as clavulanic acid. [(4)]

Klebsiella pneumonia is Gram-negative bacilli that is one of the member of *Enterobacteriaceae* that are non-lactose fermenters. It causes clinical infections in all age groups of patients and without gender specifications. Infections caused by *Klebsiella pneumonia* ranges from pneumonia, urinary tract infections, wound infections, neonatal sepsis and meningitis. [(5)]

Currently, there are several methods for the confirmatory detection of ESBL. These are the disc diffusion method, the minimum inhibitory concentration, the use of semi-automated, automated methods and molecular methods like the polymerase chain reaction (PCR).The double disc synergy (DDST), Etest method for detecting minimum inhibitory concentration and the PCR are widely used in developed countries for routine detection of ESBLs. [(6)]

The United States Clinical and Laboratory Standards Institute (CLSI) and the United Kingdom Health Protection Agency (HPA) have published guidelines for ESBL detection in *Enterobacteriacea*e specifically for *Escherichia coli*, *Klebsiella species* and *Proteus species*. When these guidelines are followed, they have a high sensitivity of detecting ESBL producing strains of up to 96% and specificity of 98%. [(7, 8)]

However, there is no regional or national guidelines for the developing countries.
We set out to compare two phenotypic methods for the confirmatory testing of ESBL in *Klebsiella pneumoniae* to identify an easy and efficient method for their laboratory diagnosis.

MATERIALS AND METHODS

***Study area*:** The study was conducted in the Department of Medical Microbiology and Parasitology of University of Maiduguri Teaching Hospital (UMTH).
Study design: Descriptive, cross-sectional study.
***Study population*:** Hospitalized patients of UMTH Maiduguri regardless of age or sex from whose specimen *Klebsiella pneumoniae* was isolated.
Inclusion criteria: Any hospitalized patient from UMTH in whose specimen *Klebsiella pneumoniae* was isolated provided he consented.
Exclusion criteria: Hospitalized UMTH patients from whose specimen yielded isolates other than *Klebsiella pneumoniae*.
Study period: The study was conducted over six-month period from 01/01/2014 to 31/06/2014
Data collection: We collected data on sociodemographic factors of gender, age, educational level, occupation, tribe as well as ward of admission and specimen type.
Microbiologic methods: We collected patient's samples comprising of swabs,

urine, blood, pus and cerebrospinal fluid from admitted into various units of UMTH. We screened this samples to isolate *Klebsiella pneumoniae* using standard microbiologic methods of culture, Gram staining and biochemical analysis using Microbact Gram negative identification kit. [(9)]

All confirmed *Klebsiella pneumoniae* isolates were screened for ESBL enzyme using the clinical and laboratory standard institute (CLSI) breakpoints. [(7)] Suspected ESBLs producers were subjected to confirmation using two phenotypic methods of double disk synergy as well as Etest method for MIC determination. The double disk synergy method utilizes ceftazidime (30 µg), cefotaxime (30 µg) and amoxicillin (20µg) / clavulonate (10µg): [augmentin; 30 µg]). The Etest method for MIC determination was done using cefotaxime and cefotaxime + amoxicillin-clavulanic acid).

Multiplex polymerase chain reaction (PCR) method that utilizes the most prevalent genes of TEM, SHV and CTX-M in our environment was considered as a gold standard for confirmation. We compared the two methods with the gold standard to determine their sensitivity, specificity and predictive value positive.

Ethical consideration: The study protocol was reviewed and approved by the ethical review board of UMTH.

Statistical analysis: Data was entered into Microsoft Excel. Verification was done using double data entry. Results were presented as Tables and Figures where appropriate. We used statistical tool in Microsoft Excel to calculate sensitivity, specificity, Predictive value positive and predictive value negative for the two methods as compared with the gold standard.

RESULTS

A total of 178 patients were recruited during the study period. There were 103 males, with a male to female ratio of 1.4:1. The sociodemographic is as shown in Table 1. The age range of the patients ranges from 2 weeks to 69 years with a mean age of 28 years and standard deviation of 14 years. Adults greater than 24 years were found to have the greatest burden of *Klebsiella pneumoniae* of 78(41.6%). Majority of our patients; 97(54.5%) had no formal education.

Table 1: Socio-demographic Characteristics of the respondents.

Variables	Frequency	Percentage
Gender (n=178)		
Male	10357.9	
Female	7542.1	
Age (n=178)		
Children (0-14 years)	46	25.8
Young Adults (15-24 years)	58	32.6
Adults (>25 years)	78	41.6
Educational Level (n=178)		
Non-Formal	97	54.5
Formal	81	45.5
Occupation (n=178)		
Civil Servant	43	24.1
Not Gainfully Employed	59	33.1
Self Employed	76	42.8
Tribe (n=178)		
Kanuri	67	37.6
Babur	32	20.0
Shuwa Arab	22	12.4
Marghi	21	11.8
Hausa	19	10.7
Others	17	7.5

The highest proportion *Klebsiella pneumoniae* organisms representing; 69(38.8%) and 68(38.2%) isolates were detected from Surgical and Medical wards respectively. The distribution of the *Klebsiella pneumoniae* isolates is as shown in figure 1.

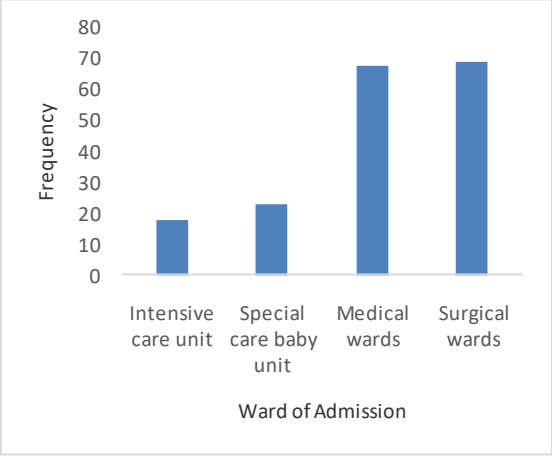

Figure 1: Distribution of the Klebsiella pneumoniae isolates based on ward of admission

The highest proportion of *Klebsiella pneumoniae* isolates of 77(43.3%) were obtained from Swabs while the least of 9(5.0%) were from Cerebrospinal fluid. The distribution of the *Klebsiella pneumoniae* isolates is as shown in Table 2.

Table 2: Distribution of the Klebsiella pneumoniae isolates based on specimen type

Specimen	Type Frequency	Percentage
Swabs	77	43.3
Urine	53	29.8
Pus	20	11.2
Blood 19	10.7	
Cerebrospinal Fluid	9	5.0

Table 3 shows the DDST method as compared with the gold standard of PCR, while Table 4 shows the Etest MIC method as compared with the gold standard of PCR. The DDST method revealed 59(33.1%) out of the 178(100%) isolates resistant with a sensitivity of 100%, specificity of 97%, positive predictive value of 93% and negative predictive value of 100%.

Using the Etest MIC, 56(31.5%) resistant isolates were identified with a sensitivity of 100%, specificity of 99%, positive predictive value of 98% and negative predictive value of 100%.

Only 55(30.9%) resistant organisms were found based on the gold standard method of multiplex Polymerase chain reaction.

Table 3: Using the DDST for confirmation of ESBL in Klebsiella pneumoniae

Polymerase Chain Reaction	**Positive**	**Negative**	**Total**
Double Disk Synergy Method			
Positive	55	4	59
Negative	0	119	119
Total	55	123	178

Table 4: Using the Etest MIC for confirmatory testing of ESBL in Klebsiella pneumoniae

Polymerase Chain Reaction	**Positive**	**Negative**	**Total**
Etest Method			
Positive	55	1	56
Negative	0	122	122
Total	55	123	178

DISCUSSION

This study provides a database for the laboratory diagnosis of ESBL producing *Klebsiella pneumoniae* from a developing country. The various methods of DDST, Etest for MIC determination and PCR have all been validated and tested in developed countries but there is dearth of study for comparative analysis in developing nations like our study area. Several studies have also reported that these various confirmatory methods may differ in their ability to detect cephalosporin resistance in the ESBL-producing strains. [(1,3,5)]

Our study revealed a marginal lower specificity with the DDST as compared to Etest MIC when both were tested against the gold standard. This implies that we tend to have some marginal high false positive results with the DDST. The finding from our study is in agreement with the work done by Zali *et al*; they reported that the clinical strains producing SHV-6 ESBL and Amp C type β-lactamase producers would not normally be detected by double disc diffusion tests. [(6)]

Another study done by Hassan *et al* to compare DDST with the CLSI based detection showed that most of the ESBL positive *Klebsiella pneumoniae* were detected by CLSI (99.5%) confirmatory test ($p<0.0001$) than DDST (67.8%). CLSI even detected ESBLs in those 69 isolates which were missed by DDST. [(10)]

The Polymerase chain reaction has been adjusted as the best method and the gold standard of diagnosis for ESBL producing organisms. Similarly, using multiplex PCR with the prevalent ESBL genes in our environment, our study was able to confirm all the isolate detected by the two methods of DDST and Etest/MIC, in addition to even ruling out the false positive isolates. Unfortunately, this method is expensive, reagent not readily available and expertise lacking from developing countries as compared to the developed countries. [(11)]

The two methods used in this study to confirm the presence of ESBLs; the double disk synergy test and the cefotaxime + cefotaxime/clavulanate MIC by epsilon test reported a seemingly better result with Etest in terms of specificity but a similar result for all of them in terms of sensitivity.

This was similar to a study done to detect ESBL in clinical isolates of

Escherichia coli from Granada, Spain using three different methods; double disk synergy method, epsilon test and Vitek-2 automated system. Out of the 62 isolates with ESBL, VITEK-2 system detected 62 (100%) while 61(98.4%) were confirmed to contain ESBLs by double disk synergy method and epsilon test. This shows that both the two methods are effective in the confirmation of ESBLs in equal ratio. [(12)]

Statistically speaking, a change in sensitivity generally has a greater effect on the predictive value of a test as comparable to a change in its specificity in terms of validity for a highly prevalent disease while the situation is reversed if we are dealing with a low prevalent disease. [(15)]

ESBL are now highly prevalent in our country as reported by Yusha'u *et al* and Mohammed *et al* from Kano and Maiduguri with prevalence of 9.25% and 33.5% respectively. [(13, 14)]

Consequently, we may tend to deduce from the above finding, sensitivity would be a better factor to use rather than the specificity in this our context and consequently, we may deduce that the validity of DDST and Etest/MIC remains the same from this study.

CONCLUSION

The two methods have high validity (high sensitivity and specificity), even though DDST had a marginal lower specificity. Although, the Etest MIC method proved to be slightly superior. In view of cost and availability of the Etest method, the DDST can be used for ESBL confirmatory testing in our clinical laboratories

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