Susceptibility Pattern Of Extended Spectrum Beta Lactamase Producing Klebsiella Pneumoniae From Clinical Isolates

I Iroha, E Amadi, A Agabus, A Oji

Citation

Abstract
The antibiotic sensitivity pattern of extended spectrum beta lactamase (ESBL) producing Klebsiella pneumoniae isolated from urine, blood and stool samples was investigated. A total of 105 clinical isolates of Klebsiella pneumoniae were isolated from urine (43), stool (38) and blood (24). Sensitivity studies were carried out using disc diffusion method by Kirby–Bauer and phenotypic characterization of ESBL was carried out using double disc synergy test (DDST). The result of the study revealed that 38 (36.2%) isolates were positive for ESBL production. 15 (34.9%) isolates were from urine, 10 (26.3%) from stool and 13 (54.2%) from blood respectively. This study therefore, do not only proclaim the presence of ESBL producing K. pneumoniae in Abakaliki, Nigeria but also emphasizes that they are multi-drug resistant.

INTRODUCTION
Extended spectrum β- lactam (ESBL) producing organisms, are now being recognized as one of the major threats to effective management of patients in medical institutions, especially in the less developed nations . Klebsiella pneumoniae, Escherichia coli and Klebsiella oxytoca have been reported by a number of workers to harbor ESBL enzyme . ESBL producing organisms are inhibited by β- lactam - β-lactamase inhibitors but are not with extended spectrum – cephalosporins . ESBL are encoded by genes on plasmids which results in easy transfer of ESBL enzymes to other bacteria species . Originally, ESBL enzymes were derived from the widespread TEM and SHV β- lactamase family, however today, over 110 derivatives of TEM β- lactamase and more than 63 derivatives of SHV β-lactamases are known . Detection of ESBL enzymes using routine laboratory susceptibility tests is often difficult and consequently, ESBL producing Klebsiella species and E. coli may falsely appear to be susceptible to newer cephalosporins . The National Committee for Clinical Laboratories Standards (NCCLS) recommends that Microbiology laboratories should report ESBL – isolates of E. coli and Klebsiella species as resistant to all penicillins and cephalosporins including cefepime and aztreonam irrespective of their individual in vitro test results . The present study was designed to determine the antibiotic susceptibility patterns of ESBL producing K. pneumoniae from different clinical specimen.

MATERIAL AND METHODS
ISOLATION OF ORGANISMS
A total of one hundred and five (105) clinical isolates of Klebsiella pneumoniae were isolated from three different clinical specimen namely, urine (43), stool (38) and blood (24) obtained from Medical Microbiology laboratory unit of Ebonyi State University Teaching Hospital (EBSUTH) Abakaliki.

SENSITIVITY STUDIES
The sensitivity studies was conducted using the Kirby and Bauer method of Sensitivity determination. Sterile Petri – dishes of Mueller Hinton agar were prepared according to manufactures specification. 0.1ml of Klebsiella pneumoniae equivalent to 0.5 McFarland standard was seeded into each of the Petri-dishes containing Mueller-Hinton agar. These were allowed to stand for 45 minutes to enable the inoculated organisms to pre-diffusion. The following antibiotics discs were aseptically placed on the surfaces of the sensitivity agar plates; ofloxacin, (15μg), gentamicin (10μg), streptomycin (5μg), ampicillin (5μg), nitrofurantoin (25μg), cephorex (15μg), augmentin (30μg), cefotaxime (30μg), ceftazidime (30μg), nalidixic acid (20μg),
Susceptibility Pattern Of Extended Spectrum Beta Lactamase Producing Klebsiella Pneumoniae From Clinical Isolates

ceftriaxone (30µg), septrin (10µg), amoxicillin (10µg), perflxacin (30µg), amoxacillin (30µg) (Oxoid, UK). These were incubated for 18 – 24 hrs at 37 C and the radial zone of inhibitions were taken. Any organism that was resistant to any of the 2\textsuperscript{nd} and 3\textsuperscript{rd} generation cephalosporins was tested for ESBL production.

DETECTION OF ESBL PRODUCTION

All isolates that was resistant to any of the 2\textsuperscript{nd} and 3\textsuperscript{rd} generation cephalosporins (ceftazidime, ceftriaxone cefotaxime) were tested for ESBL production using the double disc synergy test (DDST). Several plates of Mueller-Hinton agar were prepared and 30µg discs of ceftazidime and cefotaxime were placed 15 mm center to center from an amoxicillin – clavulanic acid disc (20:10µg ) (Oxoid UK). Inoculated media were incubated for 18 – 24 hours at 37 C. Enhanced zone of inhibition between any of the beta-lactam discs and the center disc (amoxicillin/clavulanic acid) was recorded.

RESULTS

The result of the present study showed that out of the 105 Klebsiella pneumoniae isolates, 38 (36.2%) showed evidence of ESBL production (Table 1). Isolates from blood yielded the highest percentage ESBL producers (54.2%) while stool sample gave the least (26.3%). (Table 1).

The susceptibility studies revealed a clear difference in susceptibility patterns of organisms obtained from different specimens including urine, blood and stool. ESBL producing organisms isolated from urine were susceptible to nitrofurantoin, nalidixic, ceporex, augmentin, ciprofloxacin and amikacin while isolates from blood were susceptible to nitrofurantoin, gentamicin, augmentin, ciprofloxacin, septrin and amikacin. Further, organisms isolated from stool specimen were susceptible to nalidixic acid, augmentin, ceporex, ciprofloxacin, and amikacin. It was observed that ESBL organisms from all the specimens were generally resistant to ceftazidime, cefotaxime and ceftriaxone (Table 2).

DISCUSSION

The result of this investigation shows that organisms harboring ESBL enzymes are multi-drug resistant and thus, could pose serious treatment challenges. The presence of ESBL on plasmids makes it possible for them to be easily transferred from one organism to another. Some studies have shown that floroquinolones and aminoglycosides have antimicrobial activity against ESBL organisms than other non β-lactams drugs \cite{9,10}. The overall result of the present study shows that while the prevalence of ESBL producing isolates in Abakaliki is currently not very high, it may escalate and cause a very serious public health problem, if not checked in good time. The constant use of third generation cephalosporins in the treatment of infections in Nigeria, is probably the reason for the current spread of ESBL organisms in our environment \cite{11}.

Information as regards ESBL are very uncommon in our environment and as a result, most clinicians probably don't
Susceptibility Pattern Of Extended Spectrum Beta Lactamase Producing Klebsiella Pneumoniae From Clinical Isolates

know when to test for ESBL or any preventive measures to adopt that will help to control its spread. This level of ignorance could culminate to devastating consequences. While some studies have shown that the use of β-lactam β-lactamase inhibitors such as amoxicillin-clavulanic acid and piperacillin – tazobactam in the treatment of ESBL infection is possible, others states that in vitro susceptibility testing does not predict a killing activity of β-lactam β-lactamase inhibitor 4. Also, Etrapenem antibiotic has been shown by some workers to have activity against ESBL from Klebsiella spp 12. It is vital to note, that some of these apparently efficacious drugs against ESBL producers are not readily available in Nigeria and where available, may be beyond the reach of the common man. The need to avert the further spread of this enzyme is thus emphasized.

It is interesting to note however, that the results of susceptibility studies also revealed that ESBL producing organisms were sensitive to the floroquinolones and the aminoglycosides than other non-beta lactam drugs. This probably indicates that they could be a drug of choice in treating infections caused by ESBL producing organisms, particularly Klebsiella pneumoniae, in our environment, at least at the present time.

In conclusion, the results of our work shows that ESBL producing Klebsiella pneumoniae isolated from different clinical specimens (blood, urine and stool) showed differences in their rate of antimicrobial susceptibility. Although the prevalence rates of ESBL in our study was not too high, it is hereby suggested that an antibiotic prescription formular should be develop in hospitals/clinics in Abakaliki, Nigeria, that will guide the use of third generation cephalosporins for therapeutic purposes.

ACKNOWLEDGEMENT

The authors appreciate the vital contributions of Prof. C.O. Esimone, Prof. M.U. Adikwu and Dr. I. Aibinu, which lead to the successful completion of this work. Also appreciated are the staff of the Medical Microbiology unit, Ebonyi State University Teaching Hospital, Abakaliki, Nigeria.

References

Author Information

I.R. Iroha, M.Sc., Ph.D.
Department Of Applied Microbiology, Faculty Of Applied And Natural Sciences, Ebonyi State University

E.S. Amadi, M.Sc., Ph.D.
Department Of Applied Microbiology, Faculty Of Applied And Natural Sciences, Ebonyi State University

A.C. Agabus, B.Sc.
Department Of Applied Microbiology, Faculty Of Applied And Natural Sciences, Ebonyi State University

A.E. Oji, B.Sc.
Department Of Applied Microbiology, Faculty Of Applied And Natural Sciences, Ebonyi State University