Original Article

Prevalence of extended spectrum beta-lactamase-producing *Klebsiella* species at the University of Ilorin Teaching Hospital

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Abstract

Background: Persistent blind antibiotic treatment of patients, in resource poor nations like Nigeria, makes the prevalence of antibiotic resistance to increase sporadically. Extended spectrum beta-lactamase (ESBL) production is one of the ways by which bacteria become resistant to antibiotics. For this reason, isolation, identification, sensitivity and screening for possible resistance genes is very important before prescription, if the affected patients must receive qualitative care particularly when their condition is chronic. **Materials and Methods:** Four hundred suspected isolates of *Klebsiella* belonging to various species obtained from routine specimens such as swabs, urine, blood, and sputum from May to October 2009 were studied. The identity of all isolates obtained was biochemically analyzed. The isolates were subjected to antibiotic susceptibility testing using modified Kirby–Bauer method and ESBL production was phenotypically determined using double disc synergy test for laboratory detection and reporting of bacteria by CLSI method. **Results:** Ninety-eight (24.5%) *isolates* expressed ESBL. Majority of the ESBL producing isolates were from swab specimens 59 (14.75%) followed by blood culture 16 (4.0%), urine 13 (3.25%), and sputum 10 (2.5%). Sensitivity patterns of ESBL producing *Klebsiella* spp. revealed that all ware resistant to augmentin (AUG), ceftazidime (CAZ), cefotaxime (CTX), cefuroxime (CRO), cefpodoxime (CPD), and none resistant to imipenem (IMP). **Conclusion:** ESBL producing *Klebsiella* spp., were present in University of Ilorin Teaching Hospital. They are resistant to augmentin (AUG), CAZ, CTX, and CPD. Presence of ESBL in any *Klebsiella* spp. has made cephalosporins which are first line antibiotics usually given non-effective, thereby reducing the treatment options. We, therefore, suggest screening and confirmation for ESBL, in other to prevent treatment failure.

Key words: Extended spectrum beta-lactamase, *Klebsiella* spp., UITH

INTRODUCTION

Beta lactamases are enzymes that degrade the beta-lactam ring of the beta-lactam antibiotic group such as penicillin and cephalosporins. Extended spectrum beta-lactamase (ESBL) is an acquired class A beta-lactamase that hydrolyzes and confers resistance to oxyimino second and third generation cephalosporins e.g. cefuroxime (CXM), cefotaxime (CTX), ceftazidime (CAZ), and ceftriaxone (CRO). This is one group of beta-lactamases that is, found in certain species of Gram negative bacilli, usually *Klebsiela* spp., *Proteus* spp. and *Escherichia coli*.[1-3] ESBL, however, are not the sole

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beta-lactamase that confer resistance to this class of antibiotics but are the most important.[4] They occur mostly among lactose fermenting members of enterobacteriaceae such as *E. coli*, *Klebsiella* species, and *Enterobacter* species and rarely in non lactose fermenters like *Pseudomonas* aeruginosa.[5] ESBLs are clinically relevant and remain an important cause of treatment failure with cephalosporins.

Klebisiella spp. is an opportunistic pathogen that causes various illnesses such as urinary and respiratory tract infections and septicaemia[5] among others. They are Gram negative nonmotile, usually encapsulated, indole and ornithine decarboxylase negative (*Klebsiella oxytoca* is indole positive); they do not produce H2 S, produce lysine decarboxylase and are generally positive in the Voges–Proskauer. The size ranges from 0.3 to 1.0 mm in width and 0.6 to 6.0 mm in length. However, in spite of their good sensitivity to beta lactam antibiotics, there are growing concerns about increasing resistance of the organism to this same class of antibiotic.

Resistance to cephalosporins such as oxyimino beta lactams was first described in 1980 and since then a linear increase in resistance has been recorded. The mechanism of resistance involves the production of ESBLs,[6] which are encoded by transferable conjugative plasmids that often encode resistance determinants to other classes of antibiotics. ESBLs are thus clinically relevant and remain an important cause of treatment failure with cephalosporins.[7]

The prevalence of ESBL producing *Klebsiella* species among clinical isolates varies from country to country and from hospital to hospital because of different approaches for prevention and control procedures.[8] In Nigeria, the prevalence ranges from as low as 9.25% in Kano to as high as 25.2% in Enugu. However, in spite of its importance, the authors are not aware of any study on ESBL producing *Klebsiella* spp. in Ilorin, a major referral center in the North central region of Nigeria. The objective of this study is to determine the prevalence of ESBL producing *Klebsiella* spp. at the University of Ilorin Teaching Hospital, Ilorin in order to establish a baseline to facilitate further studies on the subject.

MATERIALS AND METHODS

This was a cross-sectional study conducted at the Department of Medical Microbiology and Parasitology of the University of Ilorin Teaching Hospital, Ilorin from January to December, 2009 after an approval by the Ethical Review Committee of the Hospital. The Hospital is a tertiary care facility serving as a referral center to primary and secondary care hospitals in Kwara State and the adjoining states of Kogi, Niger, Ekiti, Oyo, and Osun with an annual admission and outpatient visits of approximately 10,000 and 12,000, respectively.

During 12 months that the study lasted, all consecutive isolates of *Klebsiella* spp. from various clinical specimens sent to the department were collected for analysis. The various sources of the specimen were swabs, urine, blood, and sputum. The identity of all isolates obtained was biochemically analyzed; screening for ESBL production was by modified Kirby–Bauer method using laboratory detection and reporting of bacteria with ESBL compiled by Livermore and Woodford. *Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC25922 were used as both positive and negative control respectively. The *Klebsiella* isolates were stored on nutrient agar slants at 4°C after 18 h of incubation at 37°C as described by Cheesbrough[9] before further processing. All stored isolates were reactivated by sub cultured on MacConkey agar incubated at 37°C for 18–24 h. The identity was determined using a colonial appearance on MacConkey agar. Speciation was done by testing for indole production, the reaction on ornithine decarboxylase, lysine decarboxylase, and Voges–Proskauer. All the speciated isolates were tested against the following antibiotic discs: Amoxicillin/ clavulanic (AUG 20/10 mcg), CRO (30 mcg), CAZ (30 mcg), cefoxitin (CFX 30 mcg), CTX (30 mcg), CXM (30 mcg), and imipenem (IMP 10 mcg). Interpretation of sensitivity results was in line with Clinical and Laboratory Standards Institute standard.[10]

Extended spectrum beta lactamase detection using double disc synergy test

Mueller Hinton agar plates were aseptically prepared and 0.1 ml of bacteria suspension in normal saline equivalent to 0.5 MacFarland standard were inoculated on the surface of the Mueller Hinton agar plate using a sterile swab stick. In double disc synergy test, synergy was determined between a disc of amoxycillin/clavulanic at the center, with CTX, CAZ, CRO, CXM, and CFX placed at a distance of 20 mm apart from the center disc on the surface of the culture plate. The test organisms were presumptively considered to produce ESBL if the zone of inhibition around the test antibiotic disc were more than 5 mm toward amoxycillin/clavulanic disc. This increase occurs because the clavulanic acid present in the amoxycillin/ clavulanic disc inactivates ESBL enzymes produced by the test organism.

RESULTS

A total of 400 identified strains of *Klebsiella* belonging to various species obtained from clinical specimens such as swabs, urine, blood, and sputum were used for the study. *K. pneumoniae* constituted 259 (64.8%) of the isolates. In all, 98 isolates representing 24.5% (95% confidence interval [CI] 20.3–28.7%) were positive for ESBL enzyme. Of the *K. pneumoniae* isolates, 27.0% (95% CI 21.6–32.4%) were ESBL positive while 19.9% (95% CI 13.3–26.5%) of *K. oxytoca* isolates were ESBL positive. These are shown in Table 1. Thirty nine (55.7%) of the ESBL producing *K. pneumoniae* isolates were obtained from swab specimens compared to 71.4% of ESBL producing *K. oxytoca* obtained from the same specimen source. Other specimen sources and the relative frequencies of the ESBL producing *Klebsiella* are as shown in Table 1. Distributions of ESBL producing *Klebsiella* according to the nature of specimen are shown in Table 2.

The antibiotic sensitivity pattern is as shown in Table 3. All the isolates were resistant to amoxicillin/clavulanic acid, CAZ, and CTX, but sensitive to cefpodoxime, CFX, CXM, CRO, and IMP, but organisms inferred to have ESBLs should be reported resistant to all penicillins (except temocillin), cephalosporins (except CFX), and to aztreonam, irrespective of routine susceptibility results.[10]

DISCUSSION

In the management of bacterial infections, the beta lactam antibiotics are very important, and many empirical therapies are commenced with them based on the suspected microbial etiology of the particular clinical condition using the epidemiological pattern as a guide. Indeed, these antibiotics are the first line treatment choices in some disease conditions. Since beta lactamases destroy these antibiotics, it is important that organisms producing the enzymes are isolated, and the appropriate antibiotic (based on susceptibility pattern) should be used in order to reduce morbidity and mortality.

Prevalence of ESBL producing *Klebsiella* spp. varies worldwide. The highest prevalence is found in Latin America (44.0%) while 22.4%, 13.3%, and 7.5% are found in Asia/Pacific region, Europe, and North America, respectively.[11] The prevalence of ESBL producing *Klebsiella* in this cross sectional study is 24.5% which is comparable to the range of 20–25% that has been reported from similar studies in other parts of Nigeria.[4,11-13] and the 22.4% reported from the Asia/Pacific region by Dhillon and Clark. However, it is lower than that reported from Latin America and significantly higher than those reported from Europe and North America by Dhillon and Clark. It is also higher than the 17% reported from a similar study in India by Sarojamma and Ramakrishna.[14] These observed differences in prevalence reflect the differing burden of ESBL producing Klebsiellae in different regions of the world. Especially noteworthy is the similarity of high prevalence in many developing countries of the world compared to the developed countries in Europe and North America. Antibiotic abuse is common in developing countries; indeed, many antibiotics including the penicillins and cephalosporins are sold even without physician prescription in many parts of Nigeria and this practice could have contributed to the high prevalence of ESBL producing Klebsiellae observed in this study and previous ones. It is also possible that there is a spread of resistance vertically between *Klebsiella* spp. as a result of point mutation which may bring microevolutionary change in the known types of ESBL variable (Temoniera (TEM), Sulphydryl (SHV) and CTX) to new one like SHV 12 and TEM 63 as reported by Opal and Pop Vicas.[14] Horizontal spread to other Enterobacteriaceae that are not ordinarily ESBL producers is another possible mechanism of development of resistance.[15]

Table 1: Frequency of ESBL-producing *Klebsiella* species

Isolate	ESBL-positive, *n* (% [95% CI])	ESBL-negative, *n* (% [95% CI])
Klebsiella pneumonia	70 (27.0 [21.6-32.4])	189 (73.0 [67.6-78.4])
Klebsiella oxytoca	28 (19.9 [13.3-26.5])	113 (80.1 [73.5-86.7])
All isolates	98 (24.5 [20.3-28.7])	302 (75.5 [71.3-79.7])

CI=Confidence interval, ESBL=Extended-spectrum β-lactamases

Table 2: Distribution of ESBL-producing *Klebsiellae* according to the nature of specimen

Nature of specimen	*Klebsiella pneumonia* (*n*=70); *n* (%)	*Klebsiella oxytoca* (*n*=28); *n* (%)
Blood culture	12 (17.1)	4 (14.28)
Sputum	9 (12.85)	1 (3.57)
Swab	39 (55.7)	20 (71.4)
Urine	10 (14.28)	3 (10.7)
Total	70 (100.0)	28 (100.0)

ESBL=Extended-spectrum β-lactamases

Table 3: Antibiotic susceptibility pattern of *Klebsiella* species

Antibiotic	*Klebsiella pneumonia*; *n* (%)	*Klebsiella oxytoca*; *n* (%)
AMC	21 (30)	10 (35.7)
CAZ	50 (71.4)	20 (71.4)
CPD	70 (100.0)	28 (100.0)
CXM	70 (100.0)	28 (100.0)
CFX	70 (100.0)	28 (100.0)
CTX	70 (100.0)	28 (100.0)
CRO	70 (100.0)	28 (100.0)
IMP	70 (100.0)	28 (100.0)

AMC=Amoxycillin/clavulanic, CAZ=Ceftazidime, CPD=Cefpodoxime, CXM=Cefuroxime, CFX=Cefoxitin, CTX=Cefotaxime, IMP=Imipenem, CRO=Ceftriaxone

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Conflicts of interest
There are no conflicts of interest.

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