



Short Communication

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Production of extended spectrum beta-lactamases of urinary isolates of *Escherichia coli* and *Klebsiella pneumoniae* in Ahmadu Bello University Teaching Hospital, Zaria, Nigeria

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ABSTRACT

Multidrug resistant strains of *Klebsiella pneumoniae* and *Escherichia coli* constitute a problem in many hospitals. The antibiotic susceptibility profile and the Production of Extended Spectrum Beta-Lactamase (ESBL) of the strains of *Klebsiella pneumoniae* and *Escherichia coli* were assessed by the Kirby-Bauer technique and the modified disc diffusion technique respectively. Out of a total of 65 urinary specimens submitted to the Medical Microbiology laboratory at the Ahmadu Bello University Teaching Hospital, Shika-Zaria, Nigeria, 50 isolates consisting of 33 (66%) *Escherichia coli* and 17 (34%) strains of *Klebsiella pneumoniae* were recovered from patients suspected to have Urinary Tract Infections (UTIs). Of these 50 isolates, 15(30%) were ESBL producers, made up of 6/17 (35.3%) ESBL-positive *Klebsiella pneumoniae* isolates and 9/33 (27.3%) ESBL-positive *E. coli* isolates. The susceptibility of the ESBL-positive *Klebsiella pneumoniae* isolates to ciprofloxacin, ofloxacin and amikacin were 64.7%, 82.4% and 82.4% respectively, while the susceptibility of the ESBL-positive *E. coli* isolates were: ciprofloxacin (57.6%), ofloxacin (48.5%) and amikacin (84.8%). All (100%) of the ESBL-positive *E. coli* isolates and 3/6 (50%) of the ESBL-positive *Klebsiella pneumoniae* isolates had Multiple Antibiotic Resistance (MAR) index of greater than 0.3 which is an indication that they originated from an environment where antibiotics are frequently used. It is important to determine the prevalence and antibiotic susceptibility of ESBL-producing clinical isolates as a guide to clinicians for the chemotherapy and there should be effective infection control policies to curb their spread in the hospital setting.

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Key words: Urinary tract infection, *Klebsiella pneumoniae*, *Escherichia coli*, extended spectrum β -lactamases (ESBLs).

INTRODUCTION

Extended spectrum Beta-lactamases (ESBLs) are plasmid-mediated enzymes capable of hydrolyzing oxyiminocephalosporins, penicillins and aztreonam. The incidence of ESBL-producing strains among clinical isolates has been increasing steadily over the past years resulting in the limitation of the therapeutic options (Quale et al., 2002; Jacoby et al., 2006).

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ESBLs share highly conserved amino acid sequence with penicillin binding proteins (PBPs). They are known to attack amide bonds in the beta-lactam ring of penicillins and cephalosporins (Jiang et al., 2006).

ESBL-producing *Escherichia coli* strains are a major cause of infections in humans particularly urinary tract infections (UTIs) and they occur at a rate of 2-3 per 100 hospital admissions. These could constitute

about 35-40% of all hospital acquired infections (Paterson and Bonomo, 2005).

Most episodes of UTIs are caused by *E. coli* (85%) *Staphylococcus saprophyticus* (10%) while *Proteus* and *Klebsiella pneumoniae* account for the remaining infections.

Since the etiology of UTIs and the antibiotic susceptibility of urinary pathogens have been changing over the past years in both community and hospital-acquired infections, it is justifiable in the present study to isolate the bacterial species associated with urinary tract infections in Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Nigeria, characterize the isolates biochemically, determine their antimicrobial susceptibility profiles and the extended spectrum beta-lactamase (ESBL)-producing ability of the isolates.

MATERIALS AND METHODS

Bacterial isolates

Fifty clinical isolates were obtained from sixty-five urinary specimens, purified, identified and characterized using established microbiological methods (Cheesbrough, 2002).

Antibiotic susceptibility testing

The antimicrobial susceptibility pattern of the isolates was determined using the Kirby-Bauer-modified disc diffusion technique (CLIS, 2005). The antibiotic discs included the following: nitrofurantoin (100 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), amoxicillin (30 µg), ofloxacin (5 µg), chloramphenicol (10 µg), amikacin (30 µg) and amoxicillin/clavulanic acid (30 µg). Mueller-Hinton Agar (MHA) was prepared according to manufacturer's instructions. Sterile swab sticks were used to inoculate agar plates with the standardized suspension of the isolates (0.5 MacFarland). Using a sterile pair of forceps, antibiotic discs were evenly distributed on the inoculated plates. The plates were incubated at 37 °C for 18-24 hours. Zones of growth inhibition were observed and their diameters were measured with a metric ruler. Isolates were classified as "resistant", "intermediate" and "sensitive" according to the interpretative chart of the National Committee for Clinical Laboratory Standards (CLIS, 2005).

Test for ESBL production

Production of Extended Spectrum β-lactamases was assessed by a Kirby-Bauer-modified disc diffusion technique (disc approximation test). Pure isolates were inoculated into freshly prepared Mueller-Hinton Agar plates with the aid of sterile swabs. Three antibiotic discs, namely, cefotaxime, amoxicillin-clavulanate and ceftazidime, were firmly pressed on the medium at a distance of 15 mm apart from each other. The plates were incubated at 37 °C for 24 hours. Isolates that showed a clear extension of cefotaxime and/or ceftazidime growth inhibition zone towards the disc containing clavulanate were considered ESBL producers (Livermore and Brown, 2001).

Determination of Multiple Antibiotic Resistance (MAR) index of ESBL producers

The MAR index of the ESBL-producing isolates of *E. coli* and *K. pneumoniae* were calculated using the formula below.

$$\text{MAR Index} = \frac{\text{Number of antibiotics to which resistant}}{\text{Total number of antibiotics tested}}$$

RESULTS

Bacteriology

Fifty out of the sixty-five urinary bacterial isolates obtained from the Medical Microbiology laboratory were biochemically confirmed. Out of the 50 isolates, 33(66%) were characterized as *E. coli*, and 17(34%) were confirmed as *K. pneumoniae*.

The percentage occurrence of the clinical isolates and the proportion that were ESBL producers is shown in Table 1.

Antibiotic susceptibility testing

The susceptibility profile of the *E. coli* (n=33) and *K. pneumoniae* (n=17) are shown in Figure 1. The isolates were resistant to most of the antibiotics tested but susceptible to amikacin, ofloxacin and ciprofloxacin. The resistance profile of the ESBL-producing *E. coli* (n=9) and *K. pneumoniae* (n=6) are shown in Figure 2.

Table 1: Percentage occurrence of the clinical isolates and proportion of ESBL producers

Isolate	No/ % occurrence	ESBL production
<i>E. coli</i>	33(66)	9(27.3)
<i>K. pneumoniae</i>	17(34)	6(35.3)

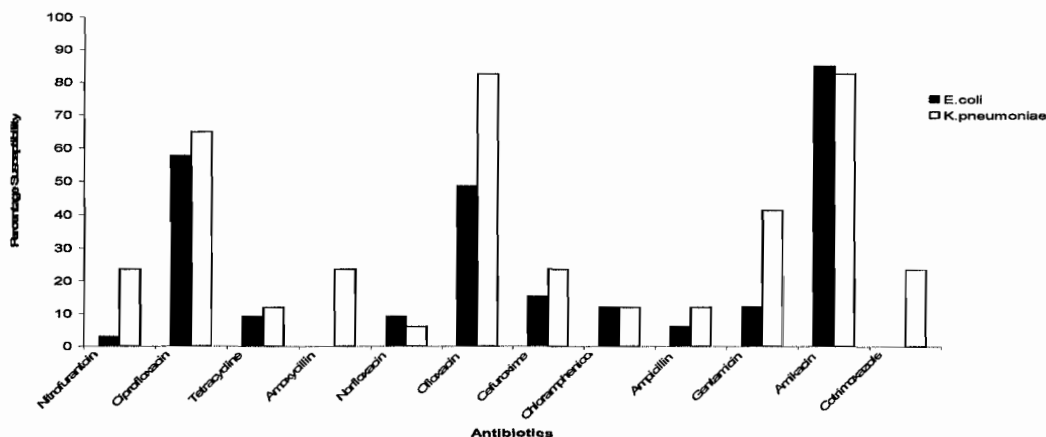


Figure 1: Percentage susceptibility of *E. coli* and *K. pneumoniae* isolates.

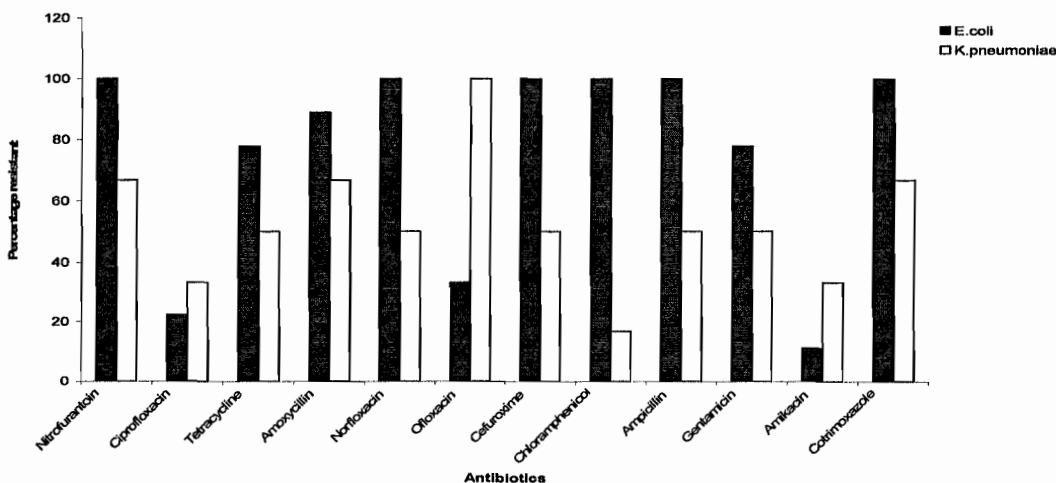


Figure 2: Resistance pattern of ESBL-producing *E. coli* and *K. pneumoniae* isolates.

The ESBL-producing isolates were resistant to most of the antibiotics tested with the ESBL-producing *E. coli* isolates being generally more resistant.

Determination of Multiple Antibiotic Resistance (MAR) index of ESBL producers

All (100%) of the ESBL-positive *E. coli* isolates and 3/6 (50%) of the ESBL-

positive *Klebsiella pneumoniae* isolates had MAR index of greater than 0.3 which is an indication that they originated from an environment where antibiotics are frequently used (Krumperman, 1983).

DISCUSSION

The incidence of infections due to organisms resistant to β -lactams has increased over the years (Burwen et al., 1994; Itokazu et

al., 1996). Most of the isolates that produce ESBLs come from patients with urinary tract infections. Such isolates have been implicated in nosocomial outbreaks (Medeiros, 1993; Livermore and Yuan, 1996). In this study, *Escherichia coli* and *K. pneumoniae* isolates were susceptible to ciprofloxacin, amikacin and ofloxacin. This agrees with the work of Quale et al (2001) that *E. coli* is susceptible to many antimicrobial agents. However, amikacin was the most effective of all the tested antibiotics. This antibiotic is not subjected to abuse because it is given intravenously. It is also expensive and less frequently prescribed (Olukoya et al., 1995; Lautenbach et al., 2001; Livermore and Brown, 2001).

Odugbemi et al (1995) observed that beta-lactamase production among enterobacteria was high. In a related study by Aibinu et al. (2003), 8 of 40 *Enterobacter* isolates produced extended-spectrum β -lactamase (ESBL) and showed high level of resistance to gentamicin, amoxicillin-clavulanic acid and trimethoprim-sulfamethoxazole. These strains were obtained from two hospitals in Lagos, Nigeria. Only four isolates transferred ESBL resistance.

β -Lactamases attack the amide lactam ring of penicillins and cephalosporins. Most of the ESBLs are mutants of the classical TEM and SHV β -lactamases types. ESBLs hydrolyse oxyimino-cephalosporins such as cefpirome, ceftazidime, cefotaxime as well as penicillins and other cephamycins. (Livermore, 1995; Braford, 2001; Quale et al., 2002).

The continued emergence of ESBL-mediated resistance in *E. coli* and *Klebsiella pneumoniae* is of great concern because those organisms are known nosocomial agents (Medeiros 1993). ESBLs producing strains of *E. coli* and *K. pneumoniae* are known to be susceptible to fluoroquinolones such as ciprofloxacin, ofloxacin and amikacin. In this study, amikacin was the most efficient antibiotic as susceptibility of the isolates was high (82.4%, 84.8% for *K. pneumoniae* and *Escherichia coli*, respectively). The resistance of the ESBL *E. coli* and *K. pneumoniae* (ESBL-EK) isolates to ciprofloxacin and ofloxacin was not too low (23.5% and 30.3%) and this shows that resistance to these antibiotics is increasing. Comparatively, in a

study by Lautenbach et al (2001), resistance to the fluoroquinolones was high (55.8%). Recent studies showed that 40-45% of ESBL-EK isolates were resistant to fluoroquinolones (Pena et al., 1995; Itokazu et al., 1996).

Treatment of infections caused by these organisms is difficult because of frequent multidrug resistance. The Multiple Antibiotic Resistance (MAR) index indicates the probable source(s) of an organism. Krumperman (1983) and Paul et al (1997) showed that MAR index greater than 0.3 indicates that such an organism must have been derived from an environment where antibiotics are often used without prescription.

The risk factors for such resistance could be aminoglycoside exposure, prolonged hospital stay, surgery, presence of a medical implant such as a urinary or arterial catheter especially in patients in intensive care units and residence in a Long Term Care Facility (LTCF), haemodialysis and gut colonization (Jacoby and Munoz-Price, 2005). Patient-to-Patient transmission of ESBL-producing *K. pneumoniae* is well documented and has been linked to the rectal colonization (Girlich et al., 2000).

In conclusion, attention to interventions to prevent the nosocomial spread of ESBL-producing *E. coli* and *K. pneumoniae* infections must be enforced in our hospitals. Restrictive antibiotic usage policies must be given priority.

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