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Extended-Spectrum Beta-Lactamase (ESBL)-producing Multidrug Resistant Enterobacteria from Commercial Poultry Feeds in Nigeria

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ABSTRACT

A total of 17 multidrug resistant (MDR) enterobacteria belonging to five genera: *Escherichia*, *Salmonella*, *Klebsiella*, *Enterobacter* and *Yersinia*; were evaluated for their potential to liberate Extended-spectrum β -Lactamases (ESBL) by the double disc synergy test. *E. coli*, *K. pneumoniae* and *S. enterica* serovar Typhi had MDR strains expressing ESBL enzyme. All MDR strains were highly resistant to amoxicillin, ceftriaxone, cotrimoxazole and gentamycin while *S. typhi* showed zero resistance to tetracycline and the fluoroquinolones (ciprofloxacin and ofloxacin). On the overall, 20.7% of the MDR strains were positive for ESBL enzyme expression with *S. enterica* ser. Typhi having the highest incidence of ESBL expression (50%) although it recorded the least MDR incidence, 6.9%. This is the first report of ESBL-producing MDR enterobacteria from poultry feed in Nigeria.

Keywords: Poultry; ESBL; Multidrug resistance; *E. coli*; *Salmonella typhi*; *Klebsiella*.

INTRODUCTION

Over the years, resistance to cephalosporins among members of enterobacteriaceae has increased mainly due to the spreading of Extended-spectrum β -Lactamases (ESBL) [1–4]. This resistance increases morbidity and mortality in infected individuals by hampering the adequate provision of effective chemotherapy therefore making treatment more costly [5–6].

The production of ESBL can be plasmid-mediated or chromosomal in origin. Plasmid-oriented ESBLs are often acquired by transfer of genetic-related information from one organism to another and it often codes for resistance determinants to other antimicrobial agents; hence, multidrug (MD) resistance is expected of ESBL-producing isolates [7–8]. However, most of these isolates have been reported susceptible to cephamycins, cabapenems and related compounds [9].

Since the introduction of cephalosporins in our health institutions, resistance by members of the enterobacteriaceae, especially *Escherichia coli*, *Klebsiella* and *Salmonella* species have been on the increase globally [7], [10–12]. Such resistance has often been derived from TEM and SHV enzymes by mutation [7], [13–14]. The prevalence of resistance to third generation cephalosporins in *E. coli* isolates from blood cultures was 4.8% in 2003, which increased to 11.7% in 2005 [15]. More recently, CTX-M-type ESBL genes have emerged in *Enterobacter* species by capture of chromosomal sequences of environmental bacteria and have undergone further genetic divergence by mutation and possibly recombination [16].

In Africa, ESBL production has been reported from different clinical sources [7], [12], [17–21] as well other sources [4]. However, information regarding ESBL production among enterobacteria isolated from poultry feeds are almost lacking especially since it has been reported that poultry feeds are a source of antimicrobial resistant zoonotic bacteria [22–24]. We could lay hands on only very few reports on ESBL incidence in poultry and poultry products in Europe [14]. Hence, the present study investigated the occurrence of multidrug resistant (MDR) enterobacteria from commercially available poultry feeds in Nigeria that were capable of producing ESBL.

MATERIALS AND METHODS

A total of 17 MDR enterobacteria obtained from commercial poultry feeds in Nigeria during our previous work [24] were used for this study. The MDR isolates represented 32.1% of all isolates obtained in that study and were of five genera: *Escherichia*, *Salmonella*, *Klebsiella*, *Enterobacter* and *Yersinia*. The isolates were purified twice on freshly prepared Salmonella-Shigella agar (SSA), Eosin-Methylene blue agar (EMB) and MacConkey agar (MCA) for this study.

All initially suspected MDR strains were subjected to a second time antimicrobial susceptibility test for confirmatory purposes using the agar-disk diffusion method [25]. Each isolate was tested against the following antibiotics on Mueller Hinton agar (Oxoid UK) – CEF: ceftriaxone (30µg), GEN: gentamycin (10µg), COT: cotrimoxazole (25µg), OFL: ofloxacin (5µg), AMX: amoxicillin (25µg), CIP: ciprofloxacin (10µg) and TET: tetracycline (30µg). Isolates resistant to four or more antibiotics were confirmed as multidrug resistant (MDR) strains and were thus subjected to double disc synergy test (DDST).

Multidrug resistant isolates were tested for ESBL production using the DDST method [26]. Cultures of MDR isolates were seeded on dry surfaced Mueller-Hinton agar (Oxoid UK) and exposed to antibiotic discs of ceftaxidime (30µg) and cefotaxime (30µg) placed at 15mm equidistance to a central disc of augmentin (30µg). Inoculated plates were incubated at 37°C for 18 – 24 hours after which the plates were read. The test isolates were phenotypically confirmed as ESBL producers if the zone of inhibition around any of the antibiotic disc increased towards the augmentin disc.

RESULTS

All isolates used in this study were confirmed as MDR strains. *E. coli*, *K. pneumoniae* and *S. enterica* serovar Typhi were the species that had MDR strains exhibiting ESBL production (Table 1, Fig. 1 and 2). *Yersinia*, *S. enteritidis* and *Enterobacter* isolates, though MDR, did not have the potential to liberate ESBL (data not shown). All MDR strains were highly resistant to AMX, CEF, COT and GEN while *S. typhi* showed zero resistance to TET and the

fluoroquinolones (CIP and OFL) (Table 1). On the overall, 20.7% of the MDR strains were positive for ESBL enzyme expression with *S. enterica* ser. Typhi having the highest occurrence of enzyme expression (50%) although it had the least MDR incidence, 6.9%.

Table 1: Antimicrobial resistance profile of three genera of enterobacteria showing ESBL production.

Isolates	Percentage (%) resistant isolates to antibiotics ^a							^b % MDR	^c % ESBL+ve
	AMX	GEN	TET	COT	CIP	OFL	CEF		
<i>E. coli</i>	93	40	40	40	7	27	67	37.9	18.2
<i>K. pneumoniae</i>	94	33	33	44	39	33	50	34.5	30.0
<i>S. enterica</i> ser. Typhi	100	50	0	50	0	0	100	6.9	50.0

^aAntibiotics: AMX, Amoxicillin; GEN, Gentamicin; TET, Tetracycline; COT, Cotrimoxazole; CIP, Ciprofloxacin; OFL, Ofloxacin; CEF: Ceftriaxone.; ^b% MDR: percentage isolates of a species showing multidrug resistance among all tested MDR enterobacteria; ^c% ESBL+ve: percentage multidrug resistant strains of a species producing ESBL



Fig. 1: *E. coli* showing ESBL production by DDST method. Ceftazidime (left disc), clavulanic acid (middle disc) and cefotaxime (right disc).



Fig. 2: *K. pneumoniae* showing ESBL production by DDST method. Ceftazidime (left disc), clavulanic acid (middle disc) and cefotaxime (right disc).

DISCUSSION

The increasing resistance to broad spectrum cephalosporins amongst enterobacteria especially *E. coli*, *Salmonella* and *Klebsiella* species predominantly due to the production of ESBLs have been reported from different countries [3–4], [12], [27–28]. In Nigeria, many reports on ESBL isolates from clinical diagnosis are available from different researchers and in different parts of the country: Kano [18], Benin [19], Lagos [17, 29], Enugu [30], and few more which we could not lay our hands on. However, data for ESBL producers from poultry feed is lacking.

In this study, we recorded ESBL production in about 20.7% of our MDR strains of enterobacteria. This data raises serious concern as it is in line with the previous reports of Ibukun *et al.* [17] who reported the occurrence of about 20.8% ESBL-producing isolates of *Klebsiella* and *E. coli* from Lagos, Nigeria, but lower than the reports of Eytayo *et al.* [29] at 32.8% and Iroha *et al.* [30] at 58.6%. However, Yusha’u *et al.* [18] and Enabulele and Orikpete [19] reported about 9% and 11% ESBL isolates, respectively, from patients in Nigeria. In 2010, [4] reported that about 19% of the enterobacteria recovered from some commercial drinks in Kano, Nigeria were ESBL producers. This trend and our present data lead us to make an assumption that there is a relatively high incidence of ESBL isolates in Nigeria. Therefore, with the emerging resistance to this class of antibiotics, infections arising from ESBL-producing bacteria pose a greater therapeutic challenge [26].

The highest incidence of MDR strains that produced the ESBL enzyme as recorded for *S. typhi* in contrast to the least expression of ESBL enzyme by the MDR strains of *E. coli* corroborate previous reports from [3, 12] but contradict the reports of [4, 20, 28]. Pokharel *et al.* [28] reported that about 0.5% of *S. enterica* serovars Typhi and Paratyphi obtained from patients’ blood in Nepal were capable of producing ESBL while Yusha’u *et al.* [4] reported that *Klebsiella* recovered from commercial drinks in Nigeria had higher ESBL positive strains than other isolated enterobacteria (*Salmonella* and *E. coli*). Ahmed *et al.* [20] on the other hand reported that *E. coli* from patients in India had about 56% ESBL-producing strains. These isolates have been reported by many authors to dominate ESBL production and are often found in most clinical samples, thus, it is suggestive that they may be regarded as ESBL production indicators since ESBL producers are associated with increased morbidity and mortality.

Conclusively, with poultry feed at the start of the food safety chain in the ‘farm-to-fork’ model, the result of this study calls for urgent intervention and caution in the use of antimicrobials employed in poultry. We have herein documented the first report on ESBL production in MDR enterobacteria obtained from poultry feeds in Nigeria and to the best of our knowledge, in Africa.

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