

Phenotypic Characterization of Extended Spectrum Beta-Lactamases in Enterobacteriaceae from Disinfected Hospital Floors of a General Hospital in Ogun State, Nigeria

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Abstract: The relevance of environmental surfaces in the conservation and dissemination of Extended spectrum beta-lactamase (ESBL) producing bacteria in hospital setting is fast becoming undisputable. Hence, the need for hygiene checks to affirm their status. This study was aimed at examining antibiotic resistance (AR) and carriage of ESBL and AmpC beta-lactamases in Enterobacteriaceae isolated from selected hospital floors in a General hospital. Swab samples were taken from the phlebotomy, children, male surgical wards, theatre and a staff common room floors post cleaning and disinfection. Isolation and biochemical characterization were done according to conventional microbiological methods. The susceptibility of isolated organisms to antibiotics was determined using disc diffusion technique. Phenotypic screening for ESBL and AmpC was done using the double disc technique. A total of 131 isolates belonging to five genera of clinical importance were isolated. *Klebsiella* spp. was most recovered genera, and from phlebotomy. Antibiotic resistance ranged between 3.8% to ciprofloxacin and 87.7% to ampicillin. Three isolates (2 *Klebsiella* and 1 *E. coli*) from the phlebotomy were resistant to 9 out of 10 test antibiotics. Sixty-seven isolates in this study were multiple drug resistant (MDR) to at least three unrelated antibiotic classes. Extended spectrum- β -lactamase activity was detected in 32 (24.4%) of the total isolates. AmpC β -lactamase was not detected in any of the isolates. Eighty and sixty percent of sampled disinfected floors were found to be reservoir of multiple-drug resistant and ESBL-producing bacteria respectively. Such hygiene failure is a serious concern for public health.

Key words: Antibiotic resistance, Enterobacteriaceae, ESBL, Hospital floor

INTRODUCTION

The contribution of environmental surfaces to the spread of hospital acquired pathogens is increasingly being reported and its relevance to hospital hygiene has gradually achieved a non-debatable status (Ajao *et al.*, 2020). The hospital floor is grouped among the non-critical environmental surfaces in the hospitals. It is nonetheless critical in the onward transfer of pathogens owing to its propensity to act as biggest receptacle for organic matter harboring diverse infectious agents. It is constantly in contact with shoe sole and most likely, a receptacle for patient vomitus and accidental spill of fecal matter (Koganti *et al.*, 2016; Rashid *et al.*, 2016). The floor is also in continuous contact with hospital cart wheels whose destinations may extend beyond the perimeters of ward, and its contribution to pathogen spread has been described in this scenario (Peretz *et al.*, 2014).

Contrary to the earlier belief that it lends little to dissemination of pathogen in the health care field, a report conducted by center for

disease control identify the reservoir nature of hospital floors and the transferability of pathogens to other parts of hospital outfit (Rutala and Weber, 2008). Similarly, studies conducted by Koganti *et al.* (2016) and Deshpande *et al.* (2017) clearly showed the ease of contamination of patient hands as well as high touched areas by a simulated non-pathogenic viral strain introduced onto hospital room's floors was reported. These studies observed a pathogen dissemination from floor to high-touch surfaces beyond the confines of patients' rooms.

In the clinical setting, a consistent upsurge in the prevalence of ESBL carrying bacteria is being reported. Enterobacteriaceae make up an important pathogen burden of hospital floor can survive on inanimate surfaces even for months and has been reported to persist sometimes in the face of sublethal disinfection. Enterobacteriaceae expressing extended-spectrum β -lactamases (ESBLs) and AmpC-type β -lactamases have emerged as an important group of pathogens linked to nosocomial outbreaks in the hospital setting (Flores-Carrero, 2017; Paduszynska *et al.*,

2019). A study conducted by Flores-Carrero *et al.* (2017) reported different variants of ESBL genes arising from nosocomial outbreak of an *Enterobacter* sp. These organisms present a formidable resistance to efficacy of most clinically relevant antibiotics and with mounting reports on their link to increasing infections and mortalities. Similar reports have also arisen from within Nigeria (Yusuf *et al.*, 2014; Olugbemiga *et al.*, 2017). Environmental cleaning and disinfection have become an integral part of preventing transmission of pathogen especially with the emergence of studies on the roles played by poorly disinfected floors on spread of resistant bacteria and in consequence, resistance genes. Many healthcare facilities, in order to reduce pathogen burden on surfaces embrace use of a differing methods of disinfection.

Recent reports however have established a multiple-approach to cleaning which require constant monitoring to affirm the integrity of cleaning and ensure compliance to standards for cleaning in health care facilities. Though hospital cleaning and hygiene goes beyond aesthetics, physical observations of some healthcare facilities in Nigeria, reflect gross neglect in adherence to expected hygiene standard required in the health care facilities. In the light of this and especially for a Nigerian healthcare system with an almost non-existence monitoring policy on hygiene standard, this study is aimed at determining the safety and status of the disinfected hospital floors as reservoirs of ESBL and AmpC, both of which are significant antibiotic resistance determinants.

MATERIALS AND METHODS

Study site

A large state-owned hospital which provides a secondary level health care service was chosen for the study. The hospital boasts of well-defined segments and wards which include the pediatrics, surgical and laboratory among others. Verbal interaction with cleaning personnel revealed each unit was

solely responsible for their choice of disinfectant, procurement and cleaning time and procedure. It also revealed no particular consistency in the usage of a particular brand. We gathered that general cleaning and disinfection take place once daily either in the morning or evening, except when there is a special need. Selected sampling points are representative of busy units known to attract traffic in the studied health care facility.

Collection of samples

In order to have a comprehensive representation of the sampled sites which mostly consisted of large areas, an approximately 15 by 15cm square area consisting of three different areas were sampled using a sterile swab sticks moistening with 0.1ml sterile normal saline solution at the point of use. Samples were taken 45-1hr post application of disinfectant to allow for adequate contact time for disinfectant action. During the course of the study cleaning was carried out by hospital personnel, business as usual. Sampling was done weekly and spanned a period of 2 months. Collected samples were placed on ice and transported to the Microbiology Laboratory, Olabisi Onabanjo University, Ago-Iwoye and analysis commenced within six hours of collection.

Bacterial isolation

The collected swab samples were excised and placed in 5ml sterile Dey Engels D/e neutralizing broth (Hi media) to encourage optimum bacterial recovery from the disinfectant solution and incubated at 37°C for 24h. A loopful of the resulting broth culture was streaked on MacConkey agar, xylose lysine deoxycholate agar and eosine methylene blue agar for isolation of bacteria. The streaked plates were incubated overnight at 35 ± 2 °C. Distinct colonies presumptive of targeted bacteria were selected and sub-cultured on fresh selective agar plates to obtain pure cultures, which were stored in glycerol stock in freezing state. Duplicate copies were maintained on nutrient agar slants to serve as working cultures.

Bacteria were identified using the biochemical test procedures (lysine decarboxylase tests, urease test, catalase tests, indole test, oxidase tests, motility, citrate tests, methyl red and Voges-proskauer tests, urease test, H₂S test, starch hydrolysis, and sugar fermentation tests) as described by Barrow and Feltham (1999).

Antibiotic susceptibility testing

Antibiotic resistance profile of isolated organisms was determined by the Kirby-Bauer disk diffusion method. Suspensions of pure isolates were prepared by transferring pure colonies to tubes of sterile normal saline which were compared to a 0.5 McFarland standard to achieve uniform turbidity. Surfaces of Mueller Hinton agar plates were then inoculated with prepared cell suspension with the aid of sterile swab sticks. Antibiotic disks impregnated with gentamicin (30µg), chloramphenicol (30µg), tetracycline (30µg), ceftazidime (30 µg), cefpodoxime (30 µg), cefotaxime (30µg), amoxicillin/clavulanic (30µg), ciprofloxacin (5µg), and ampicillin (10µg) (Oxoid Ltd, Basingstoke, UK) were immediately applied on the seeded plates. Inoculated plates were incubated at 37°C for 24hr and the diameter of inhibition zones were measured and interpreted following the CLSI criteria of zone breakpoints for Enterobacteriaceae (CLSI, 2018)

Phenotypic detection of ESBL and AmpC production

Phenotypic confirmation of the ESBL and AmpC production was performed using the combination disc test according to CLSI (2018). Commercially available MAST D68C detection kit was used. First line screening was initially carried out; isolates that were resistant to all or one of cefpodoxime, ceftriaxone and ceftazidime were found eligible to proceed for the phenotypic testing.

Disc zone size differences were used to interpret results following manufacturers guide (discs comprised of (A) cefpodoxime

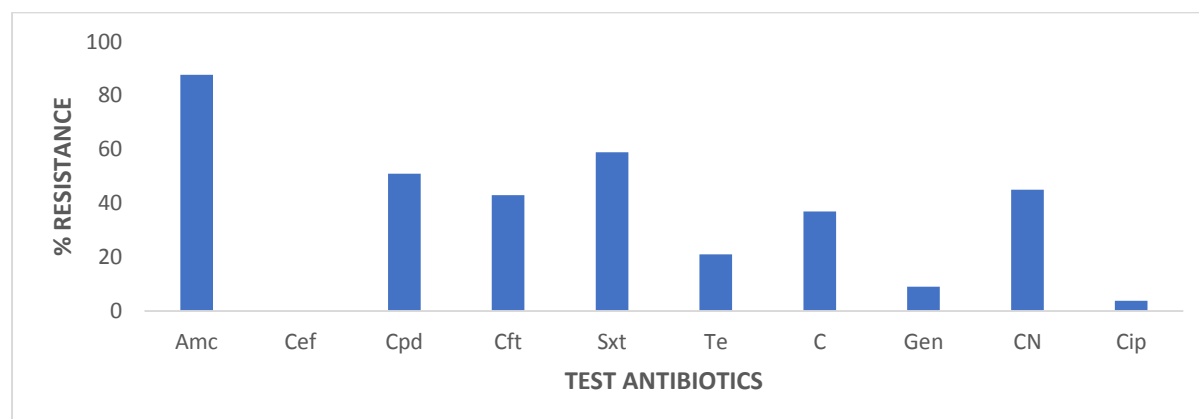
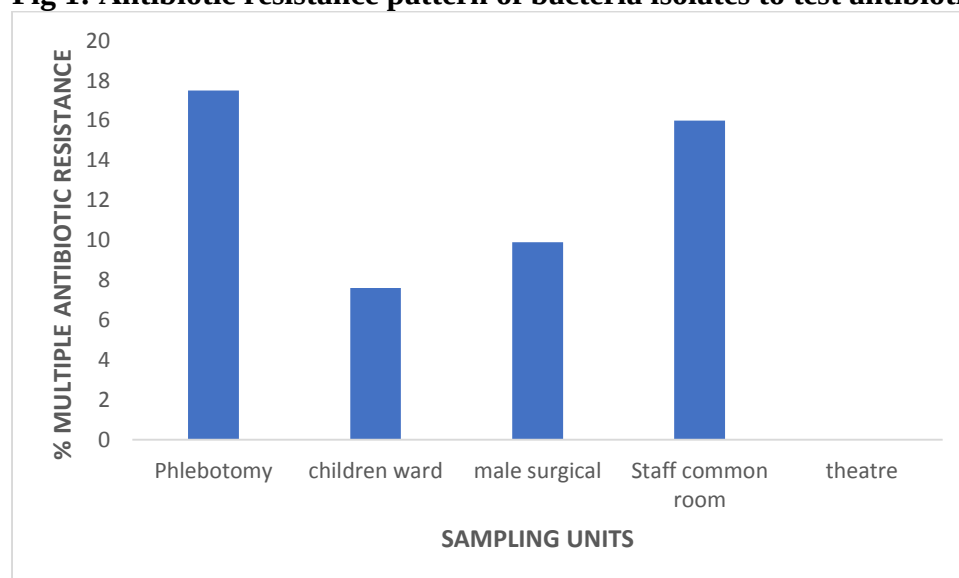
disc 10g, (B) cefpodoxime plus ESBL inhibitors, (C) AmpC inhibitors and (D) ESBL and AmpC inhibitors). Where B-A and D-C is greater or equals 5mm, and D-B and C-A is less than 5mm, such is interpreted as demonstrating ESBL activity alone. B-A and D-C is ≤ 5 mm and D-B and C-A ≥ 5 MM, org is interpreted as demonstrating AmpC activity. alone. D-C ≥ 5 mm and B-A less than 5mm was interpreted as phenotypic indication of both ESBL and AmpC production.

RESULTS

A total of 131 bacteria belonging to five genera were isolated from disinfected floors of the selected hospital units; namely; *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter* spp., *Serratia* and *Salmonella* spp. The most prevalent genera being *Klebsiella pneumoniae* and closely followed by *E. coli*. Five of the bacteria which corresponds to 3.8% of the total isolated organisms were found to be *Salmonella* spp. and the least isolated in this study (Table 1.) The susceptibility of the isolates to ten antibiotics is highlighted in Fig.1. Resistance profiles recorded against the bacterial isolates revealed varying levels of resistance to the test antibiotics used in the study, with the exception of ceftazidime, to which all of the isolates were susceptible. On the highest end is the resistance to ampicillin (n= 115/131) which corresponds to 87.7%. Fifty-six (42.3%) isolates, showed resistance to ceftriaxone, 67 (51.1%) were resistant to cefpodoxime, 77 (58.8%) showed resistance to trimethoprim/sulfamethoxazole, 28 isolates (21.4%) were resistant to tetracycline and 19 (14.5%) were resistant to trimethoprim/sulfamethoxazole (Fig 1). Forty-seven isolates representing 35.9% were resistant to one or the combination of second and third generation cephalosporins used in the study.

Table 1. Percentage isolation of bacteria from sampled floors

BACTERIA	NUMBER (%)
<i>Klebsiella</i>	44 (33.6)
<i>Salmonella</i>	5 (3.8)
<i>Enterobacter</i>	33 (25.2)
<i>Serratia</i>	8 (6.1)
<i>Escherichia coli</i>	41 (31.3)
Total	131 (100)

**Fig 1: Antibiotic resistance pattern of bacteria isolates to test antibiotics****Fig 2: Percentage multiple antibiotic resistant bacteria isolated from sampling sites**

Three isolates (2 *Klebsiella* and 1 *E. coli*) from the phlebotomy were resistant to 9 out of 10 test antibiotics. Sixty-seven (51.1%) of the total isolates in this study were found to exhibit multiple drug resistance (MDR) (to at least three unrelated antibiotic classes). By sampling sites, the highest percentage of MDR isolates were recovered from the phlebotomy (n=23) which corresponds to 17.5% of the total isolated organisms in this study. The least being from the children ward n=8 (6.1%) (Fig 2)

Table 2 is showing the number of ESBL producing organisms as revealed by the phenotypic screening method. Thirty-two (24.4%) ESBL producing Enterobacteriaceae were identified in the study and the organism

found to be most prevalent in their carriage of ESBL was *Klebsiella* spp. (n=14) closely followed by *E. coli* (n=11) and *Enterobacter* spp. (n=7) (Table 2).

Going by the site of sampling in this study, the highest number of ESBL producing organisms was recovered from the Phlebotomy room while none of the isolates from the children ward was found to exhibit ESBL activity. The detection of AmpC β -lactamase, singularly, was not detected in any

of the isolated organisms in this study; neither is the co-existence of ESBL and the Amp-C β -lactamase.

All the ESBL producers were multiple antibiotic resistant and were found to be resistant to at least 6 of the 10 test antibiotics.

Table 2. Distribution of ESBL positive Enterobacteriaceae recovered from disinfected hospital floors

Sample	ESBL(+)	Bacteria (n)	AmpC
Phlebotomy room	14	<i>E. coli</i> (3) <i>Klebsiella</i> spp. (7) <i>Enterobacter</i> spp. (2) <i>Serratia marcescens</i> (2)	Nil
Male surgical ward	11	<i>E. coli</i> (5) <i>Klebsiella</i> spp. (3) <i>Enterobacter</i> spp. (3)	Nil
Common room	7	<i>E. coli</i> (3) <i>Klebsiella</i> spp. (3) <i>Enterobacter</i> spp (1)	Nil
Theatre	Nil	Nil	Nil
Children ward	0	Nil	Nil

DISCUSSION

Over the last decades, the reputation of hospital environment as reservoir of extended spectrum β -lactamase producing bacteria including ESBL producing Enterobacteriaceae has come to stay. Though several diverse reasons contribute to spread of ESBL in healthcare facilities, transmission resulting from poorly disinfected hospital surfaces is thought to be an important contributing factor (Chaoui *et al.*, 2019). A number of studies have reported the isolation of multidrug resistant Enterobacteriaceae from environmental surfaces in the healthcare environment (Koganti *et al.*, 2016; Deshpande, 2017; Nepad, 2020). Thus, making a system of routine appraisal and feedback on the efficiency of cleaning performance very crucial.

This study found five members of the Enterobacteriaceae in different combinations from the sampled disinfected floors. The

Phlebotomy room had the highest bacteria prevalence while the least recovery of bacteria was from the children ward, it is worth of note that there was no recovery from the hospital theater. The recovery of isolates from four out of the five disinfected sampled sites could be a reflection of multiple interplay of factors but what is apparent is the inadequacy of disinfection in these floors. On the other hand, non-recovery of the target organisms from the theater floor may be as a result of a superior commitment to disinfection protocol at this unit as studies have clearly shown a good outcome of disinfection is dependent on multi factors (Deshpande *et al.*, 2017; Nepad *et al.*, 2020). In addition, the theatre appeared most clean visually, unlike the phlebotomy room and common rooms which had cemented floors with soil at most times of sampling. Others have reported the isolation of bacteria from disinfected hospital floors (Peretz *et al.*,

2014; Chaoui *et al.*, 2019) and from other hospital surfaces (Olugbemiga *et al.*, 2017). Similarly, in a study conducted by Munoz-Price *et al.* (2012) high contamination with *Staph aureus* and *Enterococcus* spp. were detectable on a phenolics-disinfected floor, suggesting a poor disinfectant action or poor application by the cleaning personnel or an interplay of both.

The high frequency in isolation of *Klebsiella* and *E. coli*, found in this study, is consistent with the study of Freeman *et al.* (2014) which investigated the ease of contamination of hospital surface with these organisms. The authors in their study, showed the ease of surface contamination and transmission of ESBL-producing *Escherichia coli* and *Klebsiella* spp. on sampled hospital surfaces by linking recovered environmental strains to contaminated human subjects. Thus, highlighting the transmissibility of these isolates in the faces of poor disinfection. The slight difference to this study was that Freeman *et al.* (2014) concentrated on high touched hospital surfaces in their own investigation.

In this study, a high level of antibiotic resistance was reported among isolated organisms in areas claimed to have been recently cleaned and disinfected. Thus, making these surfaces an under-valued reservoir of bacteria from where they can be transmitted to other frequently touched areas. Resistance to commonly prescribed antibiotics in Nigeria clinical medicine such as ampicillin, sulfamethoxazole/trimethoprim, gentamicin and third generation cephalosporin was rather high 43%-87.7%. Consequences of this ranges from prolonged hospital stay, to economic loss and complications arising from diseases caused by such resistance pathogens. Recent related study has also reported high resistance in Enterobacteriaceae from hospital surfaces (Palochoskai *et al.*, 2012; Olowo-okere *et al.* 2019). Observed resistance to the β -lactam antibiotics is particularly troubling. The resistance may have evolved and spread as a

result of the antibiotic selection pressure associated with health care facilities, as well as exposure to sublethal doses of disinfecting/cleaning substances. Moreover, the beta-lactam antibiotics are reputed as the most widely used antibiotics and by extension one of the most inappropriately used. As found in this study, high resistances to 3rd generation beta-lactams have also been reported by some authors from within Nigeria (Olowo-okere *et al.*, 2019). This is of special concern as this constitutes grave consequence to public health. Moreover, bacteria found in this study are listed among priority pathogens by the World health organization, requiring new antibiotics having mostly become resistant to common ones (WHO, 2017).

In this study, ESBL was detected in 32 isolates which accounted for 24.4% of the total isolates and recovered from three prominent units of the hospital; the Phlebotomy room, children ward and staff common room. There was however no detection of the AmpC- β -lactamase gene. Enterobacteriaceae expressing extended-spectrum β -lactamases (ESBLs) and AmpC-type β -lactamases are an important group of pathogens in the hospital setting and their relevance have been widely reported in related studies (Flores-Carrero *et al.*, 2016; Paduszynska *et al.*, 2019). However, the findings of Nahed *et al.* (2020) where bacteria including some members of the Enterobacteriaceae family were isolated from the operating theatre is not in agreement with this study as none was isolated in the theatre. In Nigeria, evidence of ESBL spread via hospital environment has been widely reported but mostly from clinical and wastewater sources (Adelowo *et al.*, 2018; Banjo *et al.*, 2020); while the few existing studies have mostly concentrated on high touched hospital surfaces adjudged to be more important in pathogen transmission (Olugbemiga *et al.*, 2017; Nwafia *et al.*, 2019). Studies as this, which investigated hospital floor for its contribution to reservoirs of ESBL and AmpC bacteria is near non-existent.

The present study, though limited to phenotypic characterization, nevertheless calls for attention to hospital floors; a rather neglected area, which may as well be a critical reservoir of ESBL-producing pathogens with grave risk to public health.

CONCLUSION

Disinfected floors of phlebotomy, staff common room and male surgical wards, and children ward were found to be reservoirs of multiple-drug resistant bacteria in this study. Of the sixty-seven MDR isolates from these sites, ESBL was detected in 32(24.4%). None

of the bacteria isolated however, harboured the AmpC beta-lactamase. Present findings generally highlight the need for healthcare facilities to pay renewed attention to floor and other hospital surfaces as they can harbor ESBL-producing bacteria. Compliance to standard hospital cleaning practices as well as adding routine microbiological examination to visual examination of hospital surfaces is advocated. There is a need for further studies in the area of molecular characterization of the resistance genes and investigation of other antibiotic resistance reservoirs in the hospital environment.

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