Extended-Spectrum Beta-Lactamase Producing Bacteria from Hospital Laboratory Equipment in Madonna Catholic Hospital, Abia State, Nigeria

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Abstract: Extended-spectrum Beta-lactamases (ESBLs) producing bacteria have been identified as a major cause of hospital-acquired infections that can have serious clinical consequences, including multiple drug resistance. This study aimed to screen hospital laboratory equipment for extended-spectrum beta-lactamase-producing bacteria. A total of sixty (60) samples were collected by swabbing various surfaces of hospital equipment using sterile swab sticks. The bacterial species were isolated and identified using standard microbiological procedures. The bacterial isolates were then subjected to an antibiotic susceptibility test on Mueller Hinton agar using the Kirby Bauer disc diffusion technique. Subsequent screening for extended-spectrum beta-lactamase production was done using the double disc synergy test. This study revealed that a total of twenty-five (25) bacterial strains were isolated from the hospital laboratory equipment, among which *Escherichia coli* 10 (40%) were the most predominant bacteria specie isolated. This was followed by *Staphylococcus aureus* 7 (28.0%) and *Salmonella* spp. 4 (16.0%), the least isolated bacteria were *Bacillus* spp. 1 (4.0%). The distribution of these isolates among the equipment showed that the incubator had the highest number and percentage of bacteria isolates (64.0%), while the least was recorded for centrifuge 3 (12.0%). The multidrug resistance profile showed that *Escherichia coli* was highly resistant to the antibiotics tested at an index rate of 0.6. Extended-spectrum beta-lactamase production was observed in *Salmonella* spp 3 (75.0%), *Escherichia coli* 2 (20.0%), and *Klebsiella* spp. 1 (33.3%) The study identified that multidrug-resistant and ESBL-producing bacteria species were present in hospital laboratory equipment and their occurrence on these equipments poses important healthcare-associated problems as they serve as a major cause of nosocomial infections. This therefore, requires strict infection control measures and careful selection of therapy in the study area to prevent the spread of these pathogens.

Keywords: Laboratory equipment, Resistance, Hospital, Bacteria, Beta-lactamase

INTRODUCTION

Antibiotic resistance has increased over the past years among clinical isolates and, as a result, is spreading rapidly worldwide (Word Health Organisation, 2014). The threat of antibiotic resistance undermines progress in health care, food production, and life expectancy. Antimicrobial resistance (AMR) is a natural phenomenon amplified by continuous and unnecessary exposure to antimicrobials (Word Health Organisation, 2014).

Extended-spectrum Beta-lactamases (ESBLs) have been identified as a significant cause of hospital-acquired infections, and they can have serious clinical consequences, including multiple drug resistance (Bharat *et al.*, 2006). The most common drug-resistant bacteria are those that produce ESBLs (Pana, 2018). These enzymes produced by these organisms can hydrolyze third-generation cephalosporins and aztreonam, however clavulanic acid inhibits them. These enzyme-producing bacteria are frequently resistant to beta-lactam antibiotics and antibiotics from other classes, creating a treatment challenge for physicians (Maina *et al.*, 2013).

Extended-spectrum beta – lactamase - producing bacteria species have worldwide distributions with varying degrees of prevalence in the community and hospitals (Legese *et al.*, 2017). The majority of ESBLs producing isolates are Gram-negative bacteria. ESBL production has become more widespread in enteric bacilli, such as *Enterobacter aerogenes, E. cloacae, Serratia marcescens, Morganella morganii, Providentia* spp., *Citrobacter freundii*, and *C. koserias*, as well as non-enteric bacilli, such as *Pseudomonas aeruginosa. Acinetobacter* spp., *Burkholderia cepacia*, and *Alcaligenes fecalis* have all been found to carry ESBLs (Sturenburg and Mack, 2003; Al-Jassser, 2006).

Infections due to ESBL production call for urgent attention for many reasons, including increased hospital costs, length of stay, treatment failure, and mortality rates (Pana, 2018).

The emergence of drug-resistant organisms in both hospitals and communities is a significant concern. Studies have revealed important details about changes in the spectrum of microbial pathogens and trends in antimicrobial resistance in nosocomial and community-acquired infections. It is critical to continue monitoring antimicrobial resistance patterns in hospitals to guide effective empirical therapy. The prevalence of ESBL-producing bacteria is rising worldwide (Tan *et al.*, 2020). In Arba Minch, Ethiopia, Aklilu *et al.* (2020) investigated the colonization rate of ESBL-producing *Enterobacteriaceae* and associated factors among hospitalized patients. Also, Aires-de-sousa (2020) evaluated the intestinal carriage of ESBL-producing *Enterobacteriaceae* at admission to a Portuguese hospital. Aside from these, Ghimire *et al.* (2017) investigated the prevalence of ESBL-producing multidrug-resistant Gram-negative bacteria from various clinical specimens in Kathmandu, Nepal. Many reports on the prevalence of ESBL-producing strain in southeastern Nigeria has not been recorded except for Oli et al. (2017), who also carried out work on the prevalence of multi-antibiotic-resistant ESBL-producing bacteria from wound and skin infections in Awka, Anambra state

Antibiotic susceptibility profile and reporting of drug-resistant strains, mainly ESBL-producing strains, would suggest the appropriate antibiotic therapy and help in awareness of misuse and overuse of antibiotics (Paterson and Bonomo, 2005). However, data on ESBL-producing bacteria from hospital laboratory equipment are limited in developing countries such as Nigeria, particularly Abia State, due to resource constraints. This study, therefore, was carried out to screen hospital laboratory equipment for extended-spectrum beta-lactamase producing bacteria at Madonna Catholic hospital, Abia State,

MATERIALS AND METHODS

STUDY AREA

This study was conducted at Madonna Catholic Hospital located at Ohokobe Afaraukwu Aba Road Umuahia. Abia State. The hospital is about 10km away from Umuahia town, and it is a health care institution designed for 200-bed spaces. The institution's services include pediatrics, laboratory analysis, cardiac clinic, ophthalmology, gynecology, outpatient rehabilitation, outpatient surgery, and Laryngological services.

Sample Collection

A total of sixty (60) swab samples were collected from various surfaces of some hospital laboratory equipment which include; an incubator, microscope, and centrifuge. The samples were collected aseptically using sterile swabs sticks wet with normal saline. The wet swab sticks were rubbed and rotated on the surfaces of the laboratory equipment and then placed in their containers, labeled, and transported in ice packs to the Michael Okpara University of Agriculture's Microbiology Laboratory in Umudike for microbiological analysis.

Isolation and purification of isolates

Direct inoculation by streak plate technique was carried out according to the methods of Cheesrough (2006). The swab sticks were used to introduce the sample into the plates and were streaked directly on the surface of the sterile culture (Mannitol salt agar, Nutrient agar, MacConkey agar, and blood agar). The culture plates were then incubated for 24 hours at 37°C for bacterial growth. The resulting colonies from the Mannitol salt agar, MacConkey agar, blood agar, and nutrient agar plates were purified by sub-culturing on nutrient agar and plates incubated for 24 hours at 37°C. After overnight incubation, the resulting discrete colonies were stored in an agar slant for further use.

Characterization of Bacterial Isolates

Morphological characteristics, Gram staining, motility test, and biochemical tests were carried out to ascertain identity of the isolate according to the methods of Cheesbrough (2006).

Motility test

The test helps detect motile and non-motile organisms. A drop of a 20 hours peptone medium culture of the test organism, with the help of a Pasteur pipette, was deposited on a clean, grease-free slide. The slide was covered with a slip and viewed under the microscope using an X40 objective lens (Cheesbrough, 2006).

Biochemical tests

The biochemical tests carried out include; the catalase test, coagulase test, citrate utilization test, methyl-Red test, Voges-Proskauer test, urease test, starch utilization, Hydrogen sulfide test, and indole test (Cheesbrough, 2006).

Antimicrobial Susceptibility Testing using Disk Agar Diffusion Method

The disc diffusion method as described by Kirby Bauer, was used in the study. Discrete colonies from 24-hour nutrient agar plates were suspended in sterile normal saline in a tube to achieve a bacteria suspension equivalent to 0.5 McFarland turbidity standards. The entire surface of the agar plates (Muller Hinton, Oxoid, UK) was then inoculated with the swab of inoculum. Antibiotics disc containing these antibiotics (Ceftriaxone, Cefotaxime, Cefuroxime, Cefixime, Imipenem, Gentamicin, Levofloxacin, Ofloxacin, Nalidixic acid, Amoxicillin, Nitrofurantoin, and Ampilox), were placed onto the inoculated plates with a sterile flame forceps and the plates incubated at 37°C for 18-24hrs. After incubation, the diameter zone of inhibition was measured with a meter rule. The drugs were interpreted as sensitive, intermediates, or resistant, following the direction of the Clinical and Laboratory Standard Institute. The zone of inhibition ≤27mmfor cefotaxime was considered a potential ESBL producer (CLSI, 2020).

Phenotypic ESBL Confirmation using Double Disc Synergy Test (DDST)

The isolated colonies were inoculated in nutrient broth at 37°C for five hours. The turbidity was adjusted to 0.5 McFarland standards, and lawn culture was made on Mueller-Hinton agar using a sterile swab. An Amoxicillin and Clavulanic acid disc (20/10ug) was placed in the center of the plate. Ceftriaxone (30ug), ceftazidime (30ug), and aztreonam (30ug) were positioned on both sides of the Amoxicillin and Clavulanic acid with a 15 mm center to center distance to the centrally located disc. Overnight at 37°C, the plate was then incubated. Extended-spectrum B-Lactamase production was interpreted as positive if the 3rd-generation cephalosporin disc inhibition was increased towards the Amoxicillin (≥5mm) disc or if none of the discs were inhibitory alone. Still, bacterial growth was inhibited when the two antibiotics were diffused together (CLSI, 2020).

Data analysis

Data were analyzed using simple percentages and presented using tables.

RESULTS

The bacteria isolated from the hospital laboratory equipment were identified as *Bacillus* spp., *Staphylococcus aureus*, *Escherichia coli, Salmonella* spp, and *Klebsiella* spp. (Table 1).

Table 2 shows the distribution and percentage occurrence of bacterial isolates from the hospital laboratory equipment. A total of twenty-five (25) bacterial strains were isolated from the hospital laboratory equipment, including *Bacillus* spp. 1 (4.0%), *Staphylococcus aureus* 7 (28.0%), *Escherichia coli* 10 (40.0%), *Salmonella* spp. 4 (16.0%) and *Klebsiella* spp 3 (12.0%). Among the hospital laboratory equipment investigated for the presence of bacteria contaminants; the incubator had the highest number and percentage of bacteria isolates, 16 (64.0%), followed by microscope 6 (24.0%), while the least was recorded for centrifuge 3 (12.0%).

Antibiotic sensitivity and resistance pattern of the bacteria isolates from the hospital laboratory equipment showed varying percentages of sensitivity, intermediate, and resistance to the tested antibiotics. Ofloxacin (5ug), Gentamicin (10ug), and Levofloxacin (5ug) were the most effective antibiotics tested against bacterial isolates from all the sample sources. Meanwhile, *Escherichia coli* isolate is the only bacteria species resistant to about eight (8) antibiotics tested (Table 3).

In the Multiple Antibiotics Resistance Index (MARI), it was observed that *Escherichia coli* isolated had the highest level of multidrug resistance index at 0.6. In contrast, the least multidrug index was recorded against *Staphylococcus aureus* at 0.3 (Table 4).

The phenotypic confirmatory test for ESBL-producing isolates based on the double disc synergy test (DDST) was shown in Table 5. A total of twenty-five (25) bacteria isolates were screened for ESBL production using CLSI breakpoint. Only nineteen (19) of the isolates revealed diameters of zones of inhibition (<25mm) when tested against cefotaxime (25ug) antibiotics which categorize them as potential ESBL producers. The confirmatory ESBL test of the isolates based on the double disc synergy test (DDST), only six (6) isolates were confirmed as potential ESBL producers. *Staphylococcus aureus* and *Bacillus* sp did not show ESBL production.

Table 1: Gram Reaction and Biochemical of Bacterial Isolates from the hospital laboratory equipment

S/N	Gram Reaction	Cell Arrangement	Catalase	Oxidase	Coagulase	Indole	Citrate	Motility	Methyl Red	Voges-P	H_2S	Urease	Glucose	Lactose	Probable Organisms
1	-	Short Rod	+	-	-	+	-	+	-	-	-	-	AG	AG	*Escherichia coli*
2	-	Short Rod	+	-	-	-	+	+	-	-	-	-	A	A	*Klebsiella* sp
3	+	Cocci	+	+	+	+	-	-	+	+	+	+	AG	AG	*Staphylococcus aureus*
4	-	*Bacilli* Shape	+	-	-	-	+	+	-	+	-	-	A	A	*Bacillus* sp
5	-	Rod Shape	+	-	-	-	+	+	+	-	+	-	AG	NAG	*Salmonella* sp

Key: - = Negative + = Positive, H_2S = Hydrogen sulphide, AG = Acid and Gas Production, A = Acid Production, NAG = No Acid Production

Isolates	Colonial Morphology
Escherichia coli	Pink-colored, circular, slightly raised, smooth colonies on MacConkey agar
Klebsiella sp	Very dense, mucoid, pink colonies on MacConkey Agar
Staphylococcus aureus	Golden yellow colonies on Mannitol salt agar
Bacillus sp	Fuzzy white circular colonies Nutrient agar
Salmonella sp	Pink with a black center raised colonies on MacConkey agar

Table 2: Distribution and Percentage Occurrence of Bacterial Isolates from the hospital laboratory equipment

Bacteria Isolates	Frequency Occurrence			No of Isolates (%)
	Incubator	Microscope	Centrifuge	
Escherichia coli	6	3	1	10 (40.0%)
***Klebsiella* sp**	2	0	1	3 (12.0%)
Staphylococcus aureus	4	2	1	7 (28.0%)
***Bacillus* sp**	1	0	0	1 (4.0%)
***Salmonella* sp**	3	1	0	4 (16.0%)
Total	16(64.0%)	6(24.0%)	3(12.0%)	25 (100%)

Key: No = Number, + = Present, - = Absent

Table 3: Susceptibility Pattern of the Bacterial Isolates from the hospital laboratory equipment (N = 25)

Bacterial Isolates	Pattern	No Tested	Number (%) Sensitivity, Resistant and Intermediatey											
			OFX	GN	NA	NF	AUG	CTX*	IMP	CRO	ACX	ZEM	LBC	CXM
Escherichia coli	S	10	9(90.0)	0(0.0)	2(20.0)	9(90.0)	0(0.0)	1(10.0)	2(20.0)	0(0.0)	3(30.0)	0(0.0)	0(0.0)	1(10.0)
	R		1(10.0)	10(100)	1(10.0)	1(10.0)	10(100)	9(90.0)	8(80.0)	9(90.0)	1(10.0)	10(100)	10(100)	9(90.0)
	I		0(0.0)	0(0.0)	7(70.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(10.0)	6(60.0)	0(0.0)	0(0.0)	0(0.0)
***Klebsiella*sp**	S	3	2(66.6)	3(100)	1(33.3)	0(0.0)	0(0.0)	1(33.3)	1(33.3)	2(66.6)	0(10.0)	1(33.3)	3(100)	0(0.0)
	R		1(33.3)	0(0.0)	2(66.6)	3(100)	3(100)	2(66.6)	1(33.3)	1(33.3)	3(100)	1(33.3)	0(0.0)	3(100)
	I		0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(33.3)	0(0.0)	0(0.0)	1(33.3)	0(0.0)	0(0.0)
S. aureus	S	7	2(28.5)	6(85.7)	5(71.4)	1(0.0)	0(0.0)	1(14.2)	5(71.4)	0(0.0)	1(14.2)	6(85.7)	5(71.4)	4(57.1)
	R		4(57.1)	1(14.2)	1(14.2)	7(100)	1(14.2)	4(57.1)	2(28.5)	7(100)	6(85.7)	1(14.2)	2(28.5)	3(42.8)
	I		1(14.2)	0(0.0)	1(14.2)	0(0.0)	6(85.7)	2(28.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(11.1)
***Bacillus* sp**	S	1	0(0.0)	1(100)	1(100)	0(0.0)	0(0.0)	1(100)	0(0.0)	0(0.0)	1(100)	1(100)	1(100)	0(0.0)
	R		1(100)	0(0.0)	0(0.0)	1(100)	1(100)	0(0.0)	1(100)	1(100)	0(0.0)	0(0.0)	0(0.0)	1(100)
	I		0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
***Salmonella* sp**	S	4	4(100)	2(50.0)	1(25.0)	2(50.0)	1(25.0)	0(0.0)	1(25.0)	1(25.0)	0(0.0)	0(0.0)	4(100)	0(0.0)
	R		0(0.0)	2(50.0)	2(50.0)	2(50.0)	3(75.0)	4(100)	3(75.0)	2(50.0)	3(75.0)	4(100)	0(0.0)	3(75.0)
	I		0(0.0)	0(0.0)	1(25.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(25.0)	1(25.0)	0(0.0)	0(0.0)	1(25.0)

Key: CXM = cefuroxime (30ug), CTX* = cefotaxime (25ug), ZEM = cefaxime(5ug), GN = gentamicin (10ug), OFX = ofloxacin (5ug), AUG = amoxicillin (30ug), ACX = ampiclox (20ug), NF = nitrofurantoin (300ug) CRO = ceftriazone (45ug), IMP = imipenem (10ug), LBC = levofloxacin (5ug), and NA = Nalidixic Acid (30ug), % = Percentage, No = Number, S = Sensitive, R = Resistant, I = Intermediate.

Table 4: Resistant Pattern and MAR index of the bacterial Isolates

Bacterial Isolate	Resistivity Pattern	MARI
Escherichia coli	CN, AUG, CTX*, IMP, CRO, ZEM, LBC, CXM	0.6
Klebsiella sp	NA, NF, AUG, CTX*, ACX, CXM	0.5
Staphylococcus aureus	NF, CRO, ACX	0.3
Bacillus sp	OFX, NF, AUG, IMP, CRO, CXM	0.5
Salmonella sp	AUG, CTX*, IMP, ACX, ZEM, CXM	0.5

Key: CXM = cefuroxime (30ug), CTX* = cefotaxime (25ug), ZEM = cefaxime (5ug), GN = gentamicin (10ug), OFX = ofloxacin (5ug), AUG = amoxicillin (30ug), ACX = ampiclox (20ug), NF = nitrofurantoin (300ug) CRO = ceftriazone (45ug), IMP = imipenem (10ug), LBC = levofloxacin (5ug), and NA = Nalidixic Acid (30ug), Multiple Antibiotics Resistance Index (MARI) = Number to which organism is resistance / Total number of antibiotics tested

Table 5. ESBL-Production based on CLSI breakpoint and Double Disc Synergy Test (DDST)

S/N	No Tested (%)	No Potential ESBL Producers (%)	ESBL Production (%)
Escherichia coli	10 (40.0)	9 (90.0)	2 (20.0)
Klebsiella sp	3 (12.0)	2 (66.6)	1 (33.3)
Staphylococcus aureus	7 (28.0)	4 (57.1)	-
Bacillus sp	1 (4.0)	0 (0.0)	-
Salmonella sp	4 (16.0)	4 (100)	3 (75.0)
Total	25	19	6

Key: No = Number, - = No ESBL Production, ESBL = Extended Spectrum Beta-Lactamase

DISCUSSION

A total of twenty-five bacteria isolates were obtained from the samples after microbiological analysis. These bacteria isolates include; *Bacillus* spp., *Staphylococcus aureus*, *Escherichia coli, Salmonella* spp, and *Klebsiella* spp. This study showed that the most predominant bacteria specie from the hospital laboratory equipment is *Escherichia coli* 10(40.0%). This was followed by *Staphylococcus aureus* 7(28.0%) and *Salmonella* spp. 4(16.0%), while the least was recorded for *Klebsiella* spp 3(12.0%). A similar observation was made by Ghimire *et al.* (2017), who reported the predominance of *E. coli* (35%) among bacterial pathogens from various clinical specimens. Similar results were also observed by Maharjan (2010) and Upadhyay (2015). According to previous investigations, drug-resistant bacteria have been detected on various surfaces, including the floor, bed frame, furniture, patients' clothes, and bed linens (Livermore and Brown, 2001). The presence of *E. coli* on the equipment may result from contaminations from fecal sources and act as vehicles for transmitting pathogens to surfaces (Hernandez-Brenes, 2002).

The prevalence of *Staphylococcus aureus* as part of the regular flora of human skin and hands, which frequently come into contact with things in the hospital environment, may have contributed to its isolation. This also suggests the likelihood of oral or nasal contamination (aerosol discharge from the mouth and nose), implying that patients' body flora may have been shed on those surfaces (Adegoke and Okoh, 2011; Komolafe and Adegoke, 2008). The present finding is low compared with the study by Mohammed *et al.* (2017) on bacterial contamination (78%) of an operating theatre in Nigeria.

In addition, Rodrigues *et al.* (2019), in a study done in Brazil to assess bacterial contamination of inert hospital surfaces and equipment in critical and non-critical care units, recorded a 94.1% prevalence rate. Habyarimana *et al.* (2020) reported a 98.53% contamination rate on electronic devices used by healthcare workers in Rwanda.

This study observed that the incubator had the highest percentage of bacteria isolates among the hospital laboratory equipment investigated for the presence of bacteria contaminants (64.0%). This could be because of the unobserved splashes from the bacteria culture, which may lead to the proliferation of the Gram-negative bacteria (as observed in the study), whose longevity is known to be enhanced by damp sites (peculiar characteristics of an incubator). In contrast, in an investigation, Silva-Sanchez et al. (2020) and Kramer et al. (2006) revealed that *E. coli* and *Klebsiella* spp. might survive for more than a year in dry environments.

The antibiotics susceptibility profile from this study revealed that Ofloxacin (5ug), Gentamicin (10ug), and Levofloxacin (5ug) were the most effective antibiotics tested against bacterial isolates from all the sample sources. The most predominant bacteria, specie *E. coli* (90%), was highly susceptible to Ofloxacin (5ug) and Nitrofurantoin (300ug). Meanwhile, this study observed high resistance of *E. coli* to the beta-lactam antibiotics, which suggests a potential ESBL producer. About 100% *Klebsiella* spp. Isolates showed resistance to the antibiotics; Nitrofurantoin (300ug), Amoxicillin (30ug), ampliclox (20ug), and cefuroxime (30ug), respectively, but an appreciable number of the isolate was highly susceptible to Gentamicin (10ug) and Levofloxacin (5ug). These findings agree with studies done in Sierra Leone (ceftazidime 62.9 %, ciprofloxacin 74.2%, gentamycin 74.3 %), demonstrating that resistance to commercially accessible and routinely used medications is increasing at an alarming rate (Leski *et al.* 2016). The high resistance rate of *Klebsiella* sp. indicates the need for the healthcare system to focus on infection management in healthcare institutions.

Similar research done in Madagascar found 100% resistance to ceftazidime and cefotaxime, Addis Ababa, cefotaxime (98%), and Turkey, cefotaxime (96%), which was more significant than a study conducted in Venezuela cefotaxime (68.7%) (Herindrainy *et al.* 2011; Desta *et al.*, 2016; Erdo-gan *et al.*, 2017; Angelin *et al.*, 2015). According to Iroha *et al.* (2009), β-lactam antibiotics are the most frequently prescribed against aerobic Gram-negative bacterial infections in Nigeria, and selective pressure exerted by the extensive use of these β-lactam drugs, most likely resulted in strains developing ESBL enzymes. On the other hand, *Salmonella sp* exhibited variable resistance to the antibiotics tested. It was recorded in this study that 100% of *Salmonella* isolates were resistant to cefotaxime (25ug) and cefaxime (5ug), respectively. *Salmonella enteric* Typhi serovar is a Gram-negative flagellated short rod of the Enterobacteriaceae family. Previous findings verified the presence of this bacterium and revealed the emergence of multidrug resistance variants (Gautam *et al.*, 2012; Gouzalez-Lopez *et al.*, 2014). This demonstrates the rise of plasmid-mediated ESBLs among Enterobacteriaceae in a hospital setting.

The multidrug resistance index of these bacterial species from the hospital laboratory equipment revealed that the high prevalence rate was coming from *Escherichia coli* (0.6), followed by *Klebsiella* sp, *Bacillus* sp., and *Salmonella* sp. at 0.5 each. A previous study has demonstrated a high multidrug-resistant index among Gram-negative isolates *Escherichia coli* and *Klebsiella* sp, similar to this present study (Tola *et al.*, 2021). Similar results was found in other investigations in Gondar, Ethiopia (93.5%) and Bahir-Dar, Ethiopia (93.1%) (Agersew *et al.*, 2013; Fantahun and Bayeh, 2009). The magnitude variation in MDR isolates might be attributable to using an antibiotic from a different class, the MDR definition, the

research time and specimen type, and the study population. This investigation also demonstrated that *Escherichia coli, Klebsiella* sp., and *Salmonella* sp. produced the majority of ESBL-producing isolates in various percentages. *Salmonella* sp., on the other hand, produced the most ESBLs (75%). Mathur *et al.* (2002) found 68.0% of *Escherichia coli* isolates to be ESBL producers, whereas Kumar *et al.* (2006) found 19.2% of *Escherichia coli* isolates to be ESBL producers. This rate of ESBL production in this study was higher than those reported in Ethiopia (38.4%) by Siraj *et al.*(2015), and in Jimma (36%) by Mulualem *et al.* (2012), but it was consistent with studies done in Jimma, Ethiopia (*K. pneumonia* 70.4%) (Siraj *et a*l.,2015).

The increasing reports of ESBLs producing bacteria in various settings including the present study area could not be unconnected with the observations of Canton et al. (2008) where they reiterated that the selection pressure produced by using 3rd generation cephalosporins has resulted in the formation of ESBLs among Gram-negative bacteria species. Most importantly, the large magnitude of ESBL may be due to a lack of antibiotic monitoring, increased antibiotic abuse, and inadequate infection control strategies. The detection of ESBL bacteria species from hospital laboratory equipment as reveled in the present study also necessitates strict adherence to aseptic procedures including frequent cleaning and sterilization of hospital equipment in the hospital settings. It is also recommended that ESBLs and other beta-lactamases be routinely detected.

CONCLUSION

The study revealed that *Escherichia coli* was observed to be the predominant bacteria isolated from the hospital laboratory equipment. There is presence of multidrug-resistant and ESBL producing bacteria species in hospital laboratory equipment in Madonna catholic hospital, Abia state, Nigeria. *Escherichia coli* showed the highest resistance to multiple antibiotics tested at the rate of 0.6, while the most elevated ESBL-producing bacteria was recorded for *Salmonella* specie 3(75.0%). These high rates require strict infection control measures and careful therapy selection in the study area. These organisms are important healthcare-associated pathogens and the primary cause of nosocomial infections, posing a threat to treatment.

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