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# SURVEY ON MULTI-DRUG RESISTANT AND EXTENDED SPECTRUM BETA LACTAMASES PRODUCING BACTERIA ON CONTACT SURFACES AT MURTALA MUHAMMAD SPECIALIST HOSPITAL, MAKKAH EYE CLINIC AND UMC ZHAHIR HOSPITAL, KANO STATE

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## ABSTRACT

The contaminated environmental surfaces are not only potential reservoirs for spread of microbial agents inside hospital but also in community. The development and evolution of antimicrobial resistance in pathogen has been reported to be one of the major issues confronting the global health community. Members of family Enterobacteriacae able to produce extended spectrum of beta lactamase which is responsible to hydrolyze the third generation of cephalosporin group antibiotics resulting treatment failure. The use of beta-lactams has increased considerably since its discovery so also resistant genes leading to Extended-Spectrum Beta-Lactamases (ESBL) mediated by the presence of blaCTX-M, blaTEM, and blaSHV genes present in most Gram-negative bacteria. Out of 300 swab samples collected, 218 (72.7%) bacteria were isolated. 158 (72.5%) was gram positive and 60(27.5%) was gram negative. A total of 96(44.0%) isolates were multidrug resistant. Antibiotics susceptibility test was performed by Kirby Bauer technique according to CLSI guidelines. Organisms that tested positive phenotypically for ESBL were subjected to PCR for molecular analysis. ESBL had a percentage rate of 11.3%. The coexistence of bla CTX-M, bla TEM and bla SHV gene was detected.

**Keywords**: Extended spectrum beta lactamases, Multi-drug resistant bacteria, Antibiotic resistant bacteria, Antibiotic sensitivity, Phenol coefficient test, Resistant genes

## INTRODUCTION

Bacterial infections caused by multi drug resistant bacteria are a growing global problem that threatens progress in the health and achievement of sustainable development goals. In LMICS, antibiotics use is increasing due to rise in income, affordable antimicrobials and the lack of stewardship in hospitals and poor control of over the counter sales leading to increase in antimicrobial drug resistance (Cherry *et al.*, 2016). MDR bacterial infections also leads to increase in morbidities characterized by additional stress, pains or functional disability of some vital body parts causing reduction in life quality (Cherry *et al.*,2016). In a health care facility, various items (animate and inanimate) can harbor infectious agents and can serve as a vehicle for transmission of nosocomial infections. Several intrinsic factors such as point mutation, gene amplification and extrinsic factors like horizontal transfer of resistant gene between bacteria within and across species by transposes, integrins or plasmids have been postulated for the development of resistance, which cannot be reduced once developed even by restricting the antibiotic usage (Saravanan and Raveendaran, 2013).

Extended spectrum beta lactamases (ESBL) are enzymes that serve as a significant resistance mechanism that impede the antimicrobial treatment of infection. ESBL have the ability to hydrolyze and cause resistance by breaking the beta lactam ring of third generation cephalosporins e.g. cefotaxime, ceftriaxone, ceftazidime) and monobactams e.g. aztreonam but not the cephamycins (e.g. cefoxitin and cefotetan) and carbapenems (e.g. imipenem, meropenem and etrapenem) (Sajjad *et al.*, 2019). These enzymes are sensitive to β-lactamase inhibitors (sulbactam, clavulanic acid, and tazobactam) (Jewoola *et al.*,2020). Infections caused by extended spectrum Beta lactamases (ESBL) producing bacteria have increasingly subjected to therapeutic limitations and patients with this infection are at high risk for treatment failure, long hospital stays, high health care cost and high mortality (Sajjad *et al.*, 2019).

## MATERIALS AND METHODS

### Sample Collection

Sterile swab stick, dipped into sterile normal saline was used to swab different surfaces and the surface of surgical equipment at the point of contact with patients. A total of three hundred swab samples was collected. Seventy five samples was collected from Makkah Eye Clinic. Seventy five samples was also collected from UMC Zhahir hospital and one hundered and fifty samples from Murtala Muhammad Specialist Hospital. Sample size was determined based on the Cochran's formula $N= Z^2 P q / d^2$ with a prevalence of 76% (Yusha'u *et al.*, 2010) and 0.05 level of error of significance.

### Ethical Clearance

Ethical approvals to carry out the study was obtained from the Ethical Clearance Committee, Kano State Ministry of Health.

### Isolation, Characterization and Bacterial Identification

Each swab was aseptically placed inside sterile test tubes containing nutrient broth and incubated at $37^0$C for 24 hours. There after the swab sticks were removed from the incubated tube and streaked on general purpose media (Nutrient agar), selective and enrichment media (Manitol salt agar, MacConkey agar). After inoculation, they were incubated at $37^0$C for 24 hours (Bassey *et al.*, 2022). Identification of bacterial isolates was done based on their Gram staining reactions into Gram positive or Gram negative cocci/ bacilli, cultural and biochemical characteristics as described by Cheesbrough (2006). The identified bacterial isolates were maintained on nutrient agar slants stored at $4^0$C in a refrigerator and subculture periodically.

### Antimicrobial Susceptibility Test

Antibiotic susceptibility test was done on Mueller-Hinton agar using the modified Kirby-Bauer disc diffusion method as outlined in the guidelines from Clinical and Laboratory Standards Institute (CLSI). After incubation the plates were

observed for zones of inhibition. Isolates was considered as sensitive or resistant to an antibiotic according to the diameter of inhibition zone interpretative chart (Clinical and laboratory standard institute, 2015).

**Clinical and Laboratory Standard Institute Breakpoint Test for ESBL Screening**

The sensitivity of standard inocula of isolates to ceftriaxone (AUF 30µg), cefotaxime (CTX 30µg) and ceftazidime (CAZ 30µg) discs was determined on Mueller Hinton Agar using Kirby Baueur method (Egwuatu *et al.,* 2022).

**Double Disc Synergy Method**

Isolates with zone diameters suspicious of ESBL production as pre-determined by the susceptibility test results (Cefotaxime: ≤25 mm, Ceftazidime: ≤22 mm, ceftriaxone: ≤25 mm, was subjected to the double disk synergy method to test for the presence of ESBL producing enzymes. 20ml of Muller Hinton agar was prepared and dispensed aseptically into a petri- dish and was allowed to solidify. The plate was swabbed by respective culture pre adjusted to 0.5 McFarland standards. A combination of amoxicillin (20µg) and clavulanic acid (10µg) was used. The combined disc was placed at the center of the inoculated plate. Cefpodoxime (10 µg) and ceftaxidime (30 µg) discs was placed on the agar at a distance of 15mm from the clavulanic acid (10 µg) and amoxicillin (30 µg) combination discs. The plates were incubated at 37ºC for 24 hours. Enhancement of the zones of inhibition of any of the cephalosporin beta-lactam antibiotic discs (cefpodoxime or ceftazidime) towards the amoxycillin/clavulanic acid disc caused by the synergy with clavulanate was taken as an evidence of ESBL production by potential ESBL positive organism (Adamu *et al.*,2020).

**Molecular Analysis to Determine Resistant Genes**

***DNA Extraction***

One loop full of the positive ESBL producing isolate was suspended in 2ml of sterile distilled water in an eppendorf tube. The bacterial suspension was boiled at $95^0$C in a water bath for 10 minutes and centrifuged at 13000rpm for 1 minute. The supernatant was used as DNA template for PCR (Saka *et al.,* 2020).

**Detection Of ESBL Associated Genes by PCR**

The primers for the detection of ESBLs associated genes was prepared according to the manufacturer (inqaba biotec). The three primers designed was SHV— Forward primer (5-CTTTATCGGCCCTCACTCAA -3), SHV– Reverse primer (5-AGGTGCTCATCATGGGAAAG -3), CTX-M–Forward primer (5-TTTGCGATGTGCAGTACCAGTAA -3), CTX-M– Reverse primer 5-(CGATATCGTTGGTGGTGCCATA-3), TEM–Forward (5- CGCCGCATACACTATTCTCAG AATGA -3) primer and TEM–Reverse primer (primer 5-ACGCTCACCGGCTCCAGATTTAT -3). 741.86 µl of distilled water was added to SHV forward primer , 774.44 µl was added to SHV reverse primer , 731.35 µl was added to TEM forward primer, 730 µl was added to TEM reverse primer, 778.5 µl was added to CTX-M forward primer and 761.99 µl of distilled water was added to the reverse primer. Dilution of 1:10 (10µl of stoke primer with 90µl of DNA and RNA free distilled water) of all the primers was prepared seperately as the finial working primer. The total of 6.5 µl of the primer mix was prepared by adding 1.75µl of ultrapure water, 0.5µl SHV— Forward primer (5-CTTTATCGGCCCTCACTCAA -3) + 0.5µl SHV– Reverse primer (5-AGGTGCTCATCATGGGAAAG -3) + 3.25 µl master mix + 1 µl DNA extract. The same procedure was carried out for CTX-M. The Eppendorf tubes containing CTX-M–Forward primer 0.5µl (5-TTTGCGATGTGCAGTACCAGTAA -3) + 0.5µl CTX-M–Reverse primer 5-(CGATATCGTTGGTGGTGCCATA-3) + 3.35 µl mastermix + 1.75µl of ultrapure water + 1 µl DNA extract. For the TEM primer mix , Eppendorf tubes containing 6.5µl of the reaction mixture was used for the PCRs (1.75 µl of water + 3.25 µl Mastermix + 1µl of DNA extract + 0.5µl TEM– Forward (5-CGCCGCATACACTATTCTCAGAATGA -3) primer + 5µl TEM–Reverse primer (5-ACGCTCACCGGCTCCAGATTTAT -3). The ultrapure PCR cycling conditions are as follows: initial denaturation for 15 seconds at 95°C, 30 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 1 minute 30 seconds, elongation at 72°C for 2 minutes and final elongation at 72°C for 10 minutes. PCR thermal cycler was used to run the PCR cycles. The post amplification products were analysed using 2% agarose gel electrophoresis. Gel Doc XR+ Imaging system was used in viewing the gel after exposure to UV light (Saka *et al.,* 2020).

**RESULTS AND DISCUSSION**

**Distribution of Organisms Isolated**

Out of 300 samples collected, 218 (72.7%) bacteria was isolated. 158 (72.5%) was gram positive and 60(27.5%) was gram negative. Coagulase negative *Staphylococcus* (CoNS) 55 (25.2%), *Staphylococcus aureus* 48(22%), others were *Bacillus spp.* 28(12.8%), *Streptococcus pyogenes.* 27 (12.4%), *Klebsiella pneumoniae.* 19 (8.7%), *Pseudomonas aeruginosa* 14(6.4%), *Proteus mirabilis*. 16 (7.3%) and *Escherichia coli* 11 (5 %).

**Bacterial Growth on Contact Surfaces at Makkah Eye Clinic**

In Makkah eye clinic, a total of 75 samples was collected from 40 pieces of surgical equipments, 28 contact surfaces and 7 samples from the hands of health care workers. A total of 49 (22.5 %) organisms was isolated. 12 was gram negative bacteria and 37 gram positive bacteria (Table 1).

**Table 1: Distribution of Bacterial Growth on Contact Surfaces at Makkah Eye Clinic**

| Organisms Detected | Contact Surfaces | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AKD (4) | BKD (4) | ANH (4) | BNH (4) | AF (4) | BF (4) | AS (4) | BS (4) | AF (4) | BF (4) | S (7) | D (7) | F (7) | B (7) | H (7) | Total 75(%) |
| *S. aureus* | - | - | - | - | - | - | - | - | - | - | 2 | 2 | - | 1 | 1 | 6(12.24) |
| *B. subtilis* | - | - | - | - | - | - | - | - | - | - | | 2 | 5 | 2 | - | 9(18.37) |
| *S. pyogenes* | - | - | - | - | - | - | - | - | - | - | 4 | 2 | - | 4 | 1 | 11(22.45) |
| CoNS | - | 1 | | | | | | 1 | | | 2 | 3 | 2 | 2 | 3 | 14(28.57) |
| *P. aeruginosa* | - | - | - | - | - | - | - | - | - | - | - | - | 1 | 2 | - | 3(6.12) |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *K. pneumoniae* | - | - | - | - | - | - | - | - | - | - | - | 1 | - | 2 | - | 3(6.12) |
| *P. mirabilis* | - | - | - | - | - | - | - | - | - | - | - | 1 | 1 | - | - | 2(4.08) |
| *E. coli* | - | - | - | - | - | - | - | - | - | - | - | | | - | 1 | 1(2.04) |
| Total | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 8 | 11 | 9 | 13 | 6 | 49(100) |

Keys**:** AKD- Kidney dish after surgery, BKD- Kidney dish before surgery, ANH- Needle holder after surgery,BNH- Neeedle holder before surgery, AF- Forcep after surgery, BF-Forcep before surgery, AS- Scissors after surgery, SS- Scissors before surgery,S- Sphygmomanometer, D- Door handle, F- floor, B- Bed, H- hands of health care workers.

**Bacterial Growth on Contact Surfaces at Murtala Muhammad Specialist Hospital**

In Murtala Muhammad Specialist hospital, a total of 150 samples was collected from 72 pieces of surgical equipments, 56 contact surfaces and 12 samples from the hands of health care workers. A total of 111(50.9 %) organisms was isolated. 32 isolates was gram negative bacteria and 79 isolates gram positive bacteria (Table 2).

**Table 2: Distribution of Bacterial Growth on Contact Surfaces at Murtala Muhammad Specialist Hospital**

| **Organisms Detected** | **Contact Surfaces** | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **AKD (8)** | **BKD (8)** | **ANH (8)** | **BNH (8)** | **AF (8)** | **BF (8)** | **AS (8)** | **BS (8)** | **AF (8)** | **BF (8)** | **S (14)** | **D (14)** | **F (14)** | **B (14)** | **H (14)** | **Total 150(%)** |
| *S. aureus* | - | - | - | - | - | - | - | - | - | - | 2 | 6 | 4 | 8 | 9 | 29(26.13) |
| *B. subtilis* | - | - | - | - | - | - | - | - | - | - | 2 | 3 | 2 | 2 | 2 | 11(9.91) |
| *S. pyogenes* | - | - | - | - | - | - | - | - | - | - | 6 | 3 | 2 | - | - | 11(9.91) |
| CoNS | 1 | 1 | - | - | - | - | - | - | - | - | 7 | 5 | 6 | - | 5 | 25(22.52) |
| *P. aeruginosa* | 1 | - | - | - | - | - | - | - | - | - | - | - | 2 | 3 | - | 6(5.41) |
| *K. pneumoniae* | 2 | - | - | - | - | - | - | - | - | - | - | 1 | 2 | 2 | 4 | 11(9.91) |
| *P. mirabilis* | 1 | - | - | - | - | - | - | - | - | - | - | 1 | 4 | 4 | - | 10(9.01) |
| *E. coli* | - | - | - | - | - | - | - | - | - | - | 1 | 2 | 1 | 2 | 2 | 8(7.21) |
| Total | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 18 | 21 | 23 | 21 | 22 | 111(100) |

Keys**:** AKD- Kidney dish after surgery, BKD- Kidney dish before surgery, ANH- Needle holder after surgery,BNH- Neeedle holder before surgery, AF- Forcep after surgery, BF-Forcep before surgery, AS- Scissors after surgery, SS- Scissors before surgery,S- Sphygmomanometer, D- Door handle, F- floor, B- Bed, H- hands of health care workers.

**Bacterial Growth on Contact Surfaces at UMC Zhahir Hospital**

In UMC Zhahir hospital, a total of 75 samples was collected from 40 pieces of surgical equipments, 28 contact surfaces and 7 samples from the hands of health care workers. A total of 58 (26.6%) organisms was isolated. 18 isolates was gram negative bacteria and 41 isolates was gram positive bacteria (Table 3).

**Table 3: Distribution of Bacterial Growth on Contact Surfaces at UMC Zhahir Hospital**

| **Organisms Detected** | **Contact Surfaces** | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **AKD (4)** | **BKD (4)** | **ANH (4)** | **BNH (4)** | **AF (4)** | **BF (4)** | **AS (4)** | **BS (4)** | **AF (4)** | **BF (4)** | **S (7)** | **D (7)** | **F (7)** | **B (7)** | **H (7)** | **Total 75(%)** |
| *S. aureus* | - | - | - | - | - | - | - | - | - | - | 2 | 2 | 3 | 3 | 3 | 13(22.41) |
| *B. subtilis* | - | - | - | - | - | - | - | - | - | - | 1 | 2 | 4 | 1 | - | 8(13.79) |
| *S. pyogenes* | - | - | - | - | - | - | - | - | - | - | 3 | - | - | 1 | 1 | 5(8.62) |
| CoNS | - | 1 | - | - | - | - | - | 1 | - | - | 2 | 4 | - | 3 | 5 | 16(27.59) |
| *P. aeruginosa* | - | - | - | - | - | - | - | - | - | - | - | 2 | 1 | 2 | - | 5(8.62) |
| *K. pneumoniae* | - | 2 | - | - | - | - | - | - | - | - | - | 1 | - | 2 | - | 5(8.62) |
| *P. mirabilis* | - | - | - | - | - | - | - | - | - | - | - | 1 | 3 | - | - | 4(6.99) |
| *E. coli* | - | - | - | - | - | - | - | - | - | - | - | 1 | - | - | 1 | 2(3.45) |
| Total | - | 3 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 8 | 13 | 11 | 12 | 10 | 58(100) |

Keys**:** AKD- Kidney dish after surgery, BKD- Kidney dish before surgery, ANH- Needle holder after surgery,BNH- Neeedle holder before surgery, AF- Forcep after surgery, BF-Forcep before surgery, AS- Scissors after surgery, SS- Scissors before surgery,S- Sphygmomanometer, D- Door handle, F- floor, B- Bed, H- hands of health care workers.

**Antibiogram of the Isolated Bacteria**

According to the current definition of multidrug resistant (MDR) bacteria, 31.25% (15/48) of *Staphylococcus aureus*, 34.55% (19/55) of coagulase negative *Staphylococcus*, 21.43% (6/28) of *Bacillus subtilis*, 25.93% (7/27) of *Streptococcus pyogenes*, 100% (19/19) *Klebsiella pneumoniae*, 50% (8/16) *Proteus mirabilis*, 100% (14/14) *Pseudomonas aeruginosa* and 57.14% (8/11) *Escherichia coli* was observerd to be MDR, that is strains that are resistant to at least three antimicrobial agents. The total of 96(44.0%) isolates were multidrug resistant.

**Table 4: Percentage Susceptability Pattern of Gram Positive Bacteria**

| Antibiotics | (ug) | *S. aureus* (n=48) | | *B. subtilis* (n=28) | | *CoNS* (n=55) | | *S. pyogenes* (n=27) | |
|---|---|---|---|---|---|---|---|---|---|
| | | R(%) | S(%) | R(%) | S(%) | R(%) | S(%) | R(%) | S(%) |
| LCB | (20) | 0(0) | 48(100) | 0(0) | 28(100) | 5(9.1) | 50(90.9) | 0(0) | 27(100) |
| GN | (10) | 2(4.2) | 46(95.8) | 2(7.1) | 26(92.9) | 10(18.2) | 45(81.8) | 5(18.5) | 22(81.5) |
| CXM | (30) | 6(18.7) | 39(81.3) | 4(14.3) | 24(85.7) | 15(27.3) | 40(72.7) | 3(11.1) | 24(88.9) |
| RD | (20) | 9(18.7) | 39(81.3) | 4(14.3) | 24(85.7) | 10(18.2) | 45(81.8) | 5(18.5) | 22(81.5) |
| CTZ | (30) | 43(89.6) | 5(10.4) | 6(21.4) | 22(78.6) | 50(90.9) | 5(9.1) | 5(18.5) | 22(81.5) |
| S | (30) | 8(16.7) | 40(83.3) | 6(21.4) | 22(78.6) | 5(9.1) | 50(90.9) | 3(11.1) | 24(88.9) |
| AZM | (10) | 10(20.8) | 38(79.2) | 4(14.3) | 24(85.7) | 14(25.6) | 41(74.5) | 7(25.9) | 20(74.1) |
| AMX | (20) | 26(50) | 24(50) | 10(35.7) | 18(64.3) | 31(56.4) | 24(43.6) | 9(33.3) | 18(66.7) |
| CPX | (10) | 6(12.5) | 42(87.5) | 2(7.1) | 26(92.9) | 8(14.5) | 47(85.5) | 7(25.9) | 20(74.1) |
| ERY | (30) | 6(18.7) | 39(81.3) | 0(0) | 28(100) | 15(27.3) | 40(72.7) | 7(25.9) | 20(74.1) |

KEYS: LCB= Levofloxacin, GN= Gentamycin, CXM= Cefuroxime, RD= Rifampine, CTZ= Ceftazidime, S= Streptomycin, AZM= Azithromycin, AMX= Amoxil, CPX= Ciprofloxacin, ERY= Erythromycin, S= Sensitive, R= Resistant

**Table 5: Percentage Susceptability Pattern of Gram Negative Bacteria**

| Antibiotics | (ug) | *K. pneumoniae* (n=19) | | *Proteus mirabilis* (n=16) | | *Esherichia coli* (n=11) | | *Pseudomonas aeruginosa* (n=14) | |
|---|---|---|---|---|---|---|---|---|---|
| | | R(%) | S(%) | R(%) | S(%) | R(%) | S(%) | R(%) | S(%) |
| LBC | (5) | 0(0) | 19(100) | 0(0) | 16(100) | 0(0) | 11(100) | 3(21.4) | 11(78.6) |
| CXM | (30) | 10(52.6) | 9(47.4) | 5(31.3) | 11(68.8) | 3(27.3) | 8(72.7) | 5(35.7) | 9(64.9) |
| ACX | (10) | 17(89.5) | 2(10.5) | 8(50) | 8(50) | 11(100) | 0(0) | 12(85.7) | 2(14.3) |
| CTX | (30) | 5(26.3) | 14(73.7) | 5(31.3) | 11(68.8) | 2(18.2) | 9(81.8) | 2(14.3) | 12(85.7) |
| IMP | (10) | 4(21.1) | 15(78.9) | 0(0) | 16(100) | 0(0) | 11(100) | 0(0) | 14(100) |
| OFX | (5) | 0(0) | 19(100) | 0(0) | 16(100) | 2(18.2) | 9(81.8) | 0(0) | 14(100) |
| GN | (10) | 5(26.3) | 14(73.7) | 6(37.5) | 10(62.5) | 8(72.7) | 3(27.3) | 10(71.4) | 4(28.6) |
| NA | (30) | 0(10.5) | 19(100) | 14(100) | 0(0) | 11(100) | 0(0) | 14(100) | 0(0) |
| ZEM | (5) | 8(42.1) | 11(57.9) | 5(31.3) | 11(68.8) | 3(27.3) | 8(72.7) | 3(21.4) | 11(78.6) |
| NF | (300) | 19(100) | 0(0) | 8(50) | 8(50) | 3(18.2) | 9(81.8) | 14(100) | 0(0) |
| CRO | (30) | 4(26.3) | 14(73.7) | 4(25) | 12(75) | 2(18.2) | 9(81.8) | 6(42.9) | 8(57.1) |
| AUG | (30) | 19(100) | 0(0) | 16(100) | 0(0) | 6(54.5) | 5(45.5) | 14(100) | 0(0) |
| CAZ | (30) | 5(26.3) | 14(73.7) | 5(31.3) | 11(68.8) | 3(27.3) | 8(72.7) | 2(14.3) | 12(85.7) |

KEYS: LBC= Levofloxacin, CXM= Cefuroxime, ACX= Ampiclox, CTX= Cefotaxime, IMP= Imipenem, OFX= Ofloxacin, GN= Gentamycin, NA= Nalidixic acid, ZEM= Cefixime, NF= Nitrofurantoin, CRO= Ceftriaxone, AUG= Amoxicillin clavulanate, CAZ= Ceftazidime, S= Sentitive, R= Resistant

**Presence of Extended Spectrum Beta Lactamase**

Out of the 60 gram negative isolates identified, 11(11.3%) ESBL positive isolates were confirmed using double disc synergy test. These 11 isolates consisted of 4 *Proteus mirabilis,* 3 *Klebsiella pneumoniae,* 2 *Pseudomonas aeruginosa* and 2 *Escherichia coli* isolates (Table 6). Four isolates were subjected to molecular analysis for confirmation of resistant genes. The coproduction of all three resistant genes SHV, TEM, CTX-M was found in 3 isolates, *Klebsiella pnuemonia, Proteus mirabelis* and *Pseudomonas aeruginosa.*

**Table 6: Distribution of the Different Extented Spectrum Beta Lactamase Producing Isolates Across the Three Hospitals**

| S/N | ISOLATE NAME | SAMPLE LOCATION | SOURCE OF ISOLATION | PHENOTYPIC PRESENCE |
|---|---|---|---|---|
| 1 | *Escherichia coli* | (FS) MMSH | Kidney dish | yes |
| 2 | *Escherichia coli* | (MS)MMSH | Floor | yes |
| 3 | *Klebsiella pneumoniae* | UMC | Door knob | yes |
| 4 | *Klebsiella pneumoniae* | (FS)MMSH | Health care workers | yes |
| 5 | *Klebsiella pneumoniae* | MEK | Health care workers | yes |
| 6 | *Proteus mirabilis* | (FS)MMSH | Hospital bed | yes |
| 7 | *Proteus mirabilis* | UMC | Hospital bed | yes |
| 8 | *Proteus mirabilis* | (MS)MMSH | Hospital bed | yes |
| 9 | *Proteus mirabilis* | (OP)MMSH | Health care workers | yes |
| 10 | *Pseudomonas aeruginosa* | (OP)MMSH | Door knob | yes |
| 11 | *Pseudomonas aeruginosa* | (MS)MMSH | Floor | yes |

Keys: (OP)MMSH=Murtala Muhammad Specialist Hospital, Ophthalmology Department, (MS)MMSH= Murtala Muhammad Specialist Hospital, Male Surgical Unit, (FS)MMSH= Murtala Muhammad Specialist Hospital, Female Surgical Unit, UMC= UMC Zhahir Hospital, MEK= Makkah Eye Clinic

**Discussion**

This study revealed high level of bacterial contamination with the prevalence of 72.67%. This was lower than the high prevalence of 84.0% in a study carried out by Essien *et al*. (2017) in Brigham Teaching Hospital in Jos, Plateau State, North Central Nigeria. However, the study by Melkam *et al.* (2021) revealed a prevalence of 71.1% which was slightly similar to this study. The overall multiple drug resistance (two and above antimicrobial classes) of the isolates in this study was 44.04%. this was slightly similar to the study carried out by Binita *et al*.(2022) who observed 42.11% MDR isolates from door handles in hospitals. Multiple-antibiotic resistance was detected in 57.7% of isolates done in a previous study by Melkam *et al.* (2021). Worku *et al.* (2018) also observed 53.8% MDR isolates in their study.The prevalence of MDR bacteria observed in this study might have emerged as a result of indiscriminatory and frequent usage of antibiotics per patient per ward. High resistance of the isolates to antibiotics may also be due to practicing self-medication, lack of diagnostic laboratory services or unavailability of guideline regarding the selection of drugs thereby which lead to inappropriate use of antibiotics (Essien *et al.*, 2017).

11 (18.3%) ESBL positive isolates were identified in this study this was similar to the study carried out by Egwuatu *et al.(*2022) who identified (21.7%) in their study on the detection of ESBL genes in formites, healthcare workers and patients. It was also similar to the report carried out by Jewoola *et al.*,(2020) who had a total rate of 25.3% from clinical isolates. In this study the co-existence of multiple bla SHV, bla CTX-M gene and bla TEM gene was detected in 3 ESBL producing isolates. This was similar to the findings of Gbolabo *et al.* (2023) who observed multiple ESBL gene ( bla SHV, bla CTX-M gene, bla OXA gene and bla TEM gene) in their. Out of the four ESBL isolates detected by phenotypic method, one isolate showed negative result. This may be due to the presence of other ESBL genes such as OXA gene. This was similar to the findings of Mohammed and Anwar (2022). Resiatance to third generatin cephalosporins is particularly worrisome when caused by ESBL, as the spread of these enzyme is plasmid mediated and can be transferred to other gram negative species. ESBL infections are concerning for many reasons, including increased hospital costs, lengths of stay and mortility rate (Sajjad *et al.*, 2019).

**CONCLUSION**

This study gives base line information on the contamination of medical equipment and inanimate objects with different species of bacterial pathogens in Murtala Muhammad Specialist Hospital, Makkah Eye Clinic and UMC Zhahir Hospital. Antimicrobial susceptibility tests revealed that the bacterial isolates were resistant to multiple antibiotics, indicating a high risk of nosocomial outbreaks due to drug-resistant bacteria. Therefore, strict infection prevention and control programs, routine sampling from patient-care equipment and inanimate objects should be implemented, along with antimicrobial-resistance surveillance and decontamination efforts. Sooner or later these antibiotics resistance genes may spread from one species to another by means of recombination and eventually lead to the evolution of newer resistant organisms. This study also established the presence and distribution of ESBL encoding genes within the hospital. Based on the results of this study, we advise that health care workers should implement good hygiene and management practices especially within the hospital environment and should be included in the study of detection of Extended spectrum beta-lactamases as this study shows that health care practitioners can act as a carrier of these enzymes from the hospital to the community.

**REFERENCES**

Adamu, Y., Ali, A. H., Timothy, B. and Mohammad, M. M.(2020). Occurrence of extended spectrum beta lactamase encoding gene among urinary pathogenic *Escherichia coli Klebsiella pneumoniae* isolates obtained from a tertiary hospital in Gombe, Nigeria. *Journal of Bioscience and Medicine*. 8:9.

Bassey, E. E., Tarh, J. E., Tu, J. U. And Ekpiken, E. S. (2022). Antibiogram profile of enteric pathogens isolated from fomites in Cross river university of technology medical center, Cabalar, Nigeria. *Annual Research and Review in Biology*. 21-36.

Binita, K. S., Birendra, P. S. Angeela K., Nirmala, B., Padam, D. M. and Sajana A.(2022).Prevalence of Extended Spectrum β-Lactamase producers (ESBLs) with antibiotic resistance pattern of gram negative pathogenic bacteria isolated from door handles in hospitals of Pokhara, Western, Nepal.*International Journal of Microbiology*. 7:19.

Cheery, L., Emi, T., Malliwan, H., Vanaporn, W., Visanm, T., Soawapak, H., Nicholas, P. J., Sharon, J. P. and Derek, L. (2016). Epidemiology and burden of multi drug resistant bacterial infection in developing countries. http://dx.doi.org/10.7554/elife.1802.001

Cheesbrough, M. (2006). District laboratory practice intropical Countries. 2nd edition, Cambridge University Press, London.

Clinical and laboratory standard institute. (2015). Performance standards for antimicrobial Susceptibility Testing. *Twenty Fifth Informational Supplement*. 25(7):1.

Egwuatu, T. O., Ishola, O. D. and Oladele, O. E. (2022).the distribution of extended-spectrum beta-lactamase genes in fomites, healthcare workers, and patients from two hospitals in Lagos State, Nigeria. *Journal of Science*. 21:2.

Essien, U. C., Sheyin, Z., Sati T., Ede, F. R. and Bigwan, E. I.(2017). Bacteria contamination on hospital surfaces in Bingham university teaching hospital Jos, North central Nigeria. *International Journal of Microbiology*.3(6):82-85.

Gbolabo, O., Motunrayo, Y. J., Olusola, O., Rita, A. O. and Olugbenga, A. O. (2023). Genotypic determination of extended spectrum beta lactamases and carbapenemase production in clinical isolates of *klebsiella pneumoniae* in southwest Nigeria. *Infectious Disease Report*. 15(3): 339-353.

Jewoola, O. O., Bode-sojobi, I. O., Ogunsola, F. T. and Okonji, P. E. (2020). High carriage rates of extended-spectrum betalactamase-producing Enterobacteriaceae in children at admission into paediatric wards of a university teaching hospital in Lagos, Nigeria. *Nigerian Postgraduate Medical Journal*. 27(2): 136-142.

Melkam, B., Matusal, M., Munira, S., Addis, A., Kalicha, B., Melat, W.,Gelila, B., Mohammed, S. and Aseer, M. (2021). Magnitude, diversity, and antibiograms of bacteria isolated from patient-care equipment and inanimate objects of selected

wards in Arba Minch General Hospital, Southern Ethiopia. *Research and Report in Tropical Medicine.* 12: 39–49.

Mohammed, A. B. and Anwar, K. A. (2022). Phenotypic and genotypic detection of extended spectrum beta lactamase enzyme in *klebsiella pneumoniae*. *Journal Pone.* 17(9): 26.

Sajjad, Y., Miaad, K. A., Reza, N., Elika, F., Reham, M., Morteza, S. Elham, J., Farokh, I and Atefah, A. (2019). Preliminary survey of extended spectrum beta lactamases (ESBLs) in nosocomial uropathogen *Klebsiella pneumoniae* in north central Iran.*Science Direct.*5(9):2349.

Saka, H. K., García-Soto, S., Dabo, N. T., Lopez-Chavarrias, V., Muhammad, B., Ugarte-Ruiz, M. (2020). Molecular detection of extended spectrum β-lactamase genes in *Escherichia coli* clinical isolates from diarrhoeic children in Kano, Nigeria. *Journal Pone.* 15(12):257–263.

Saravanan, R. and Raveendaran, V. (2013). Antimicrobial resistance pattern in a tertiary care hospital: an observational study. *Journal of Basic and Clinical Pharmacy*. 4(3): 56-63.

Worku, T., Derseh, D. and Kumalo, A. (2018). Bacterial profile and antimicrobial susceptibility pattern of the isolates from stethoscope, thermometer, and inanimate surfaces of Mizan-Tepi University Teaching Hospital, Southwest Ethiopia. *International Journal of Microbiology*. 20:1-7.

Yusha'u, M., Aliyu, H. M., Kumurya, A.S. and Suleiman, K. (2010). Prevalence of extended spectrum β-lactamases (ESBLS) among Enterobacteriaceae in Murtala Mohammed Specialist Hospital, Kano, Nigeria. *Bayero Journal of Pure and Applied Sciences*. 3(1): 169- 172.