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Antimicrobial Activities of Piperacillin-tazobactam Plus Oxyimino-cephalosporins and Gentamicin against Extensively Drug-resistant Non-carbapenemase-producing *Enterobacteriaceae* Isolated from Immunocompromised Patients in a Nigerian Hospital

Piperasilin-Tazobaktam Artı Oksimino-Sefalosporinler ve Gentamisinin Bir Nijerya Hastanesinde Bağışıklığı Yetersiz Hastalardan İzole Edilen Aşırı Derecede İlaca Dirençli Karbapenemaz Üretmeyen *Enterobacteriaceae*'ye Karşı Antimikrobiyal Aktiviteleri

Abubakar Suleiman KANKARA[1], Usman Aliyu DUTSINMA[2], Ibrahim YUSUF[2]

[1]*Federal Medical Centre, Department of Medical Microbiology, Katsina, Nigeria*
[2]*College of Natural and Pharmaceutical Sciences Bayero University, Faculty of Life Sciences, Department of Microbiology, Kano, Nigeria*

Abstract

Introduction: Antimicrobial resistance among the *Enterobacteriaceae* members has progressed from multi to extensively drug-resistant status, thereby limiting the treatment options for immunocompromised patients (ICPs). Monotherapy application has been proven unsuccessful in many cases, thereby necessitating a combination therapy for optimal treatment.

Materials and Methods: The incidence of extensively drug-resistant (XDR) pathogens among ICPs was studied and they were screened for β-lactamase production, and the effectiveness of antibiotic combination against the XDR *Enterobacteriaceae* isolated from a Federal Medical Center in Nigeria was determined using the standard Clinical and Laboratory Standards Institute methods. Checkerboard assay was used to determine the synergy between piperacillin-tazobactam (TZP)/amoxicillin-clavulanic acid (AMC) and each of ceftazidime (CAZ), ceftriaxone (CRO), and gentamicin (GN) by using fractional inhibitory concentration indices.

Results: A total of 68 *Enterobacteriaceae* members were isolates and 15 (22.1%) were XDR. Of the 68 isolates, 53.3%, 13.3%, and 0% were extended-spectrum β-lactamases (ESBL), AmpC, and carbapenemase producers, respectively. A resistance to meropenem was expressed by 37.5% in XDR *E. coli*, 60% in *K. pneumoniae*, and 100% in *Enterobacter aerogene*. Equally alarming was the colistin resistance expressed by 50% in XDR *E. coli* and 20% in *K. pneumoniae*. Mono antibiotics with favorable activities against the XDR *Enterobacteriaceae* included colistin, tigecycline, and meropenem. The synergy was observed for XDR *E. coli* and *K. pneumoniae* when TZP was combined with CAZ and CRO. No synergy was observed when AMC was combined with either CAZ or GN.

Conclusion: This study demonstrated the incidence of XDR *Enterobacteriaceae* among ICPs and suggested TZP plus CAZ or CRO as a useful treatment combination for infections due to XDR *Enterobacteriaceae*, including the co-producers of ESBL and AmpC β-lactamase.

Keywords: Extensive drug resistance, multidrug resistance, *Enterobacteriaceae*, immunocompromised patients, ESBL, AmpC, carbapenemase, Katsina, Nigeria

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Address for Correspondence/Yazışma Adresi: Ibrahim Yusuf MD, College of Natural and Pharmaceutical Sciences Bayero University, Faculty of Life Sciences, Department of Microbiology, Kano, Nigeria
E-mail: iyusuf.bio@buk.edu.ng **ORCID ID:** orcid.org/0000-0002-6629-8685
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Öz

Giriş: *Enterobacteriaceae* üyeleri arasındaki antimikrobiyal direnç, çoklu ilaç direncine veya aşırı derecede ilaca dirençli (XDR) duruma ilerlemiş, böylece bağışıklık sistemi baskılanmış hastalar (BSBH) için tedavi seçeneklerini sınırlamıştır. Monoterapi uygulamasının birçok durumda başarısız olduğu kanıtlanmıştır, bu nedenle optimal tedavi için kombinasyon tedavisi gerekir.
Gereç ve Yöntem: Bağışıklık sistemi baskılanmış hastalar arasında XDR patojenlerin insidansı incelendi, bu patojenler β-laktamaz üretimi açısından tarandı ve Nijerya'daki bir Federal Tıp Merkezi'nden izole edilen XDR *Enterobacteriaceae*'ye karşı antibiyotik kombinasyonunun etkinliği, Klinik ve Laboratuvar Standartları Enstitüsü yöntemleri kullanılarak belirlendi. Piperasilin-tazobaktam (TZP)/amoksisilin-klavulanik asit (AMC) ile seftazidim (CAZ), seftriakson (CRO) ve gentamisinin (GN) herbiri arasındaki sinerjiyi fraksiyonel inhibitör konsantrasyon indekslerini kullanarak belirlemek için Checkerboard testi kullanıldı.
Bulgular: Toplam 68 *Enterobacteriaceae* üyesi izolat ve 15'i (%22,1) XDR idi. Altmış sekiz izolatın sırasıyla %53,3, %13,3 ve %0'ı geniş spektrumlu β-laktamazlar (ESBL), AmpC ve karbapenemaz üreticileriydi. Meropeneme direnç; XDR *E. coli*'de %37,5, *K. pneumoniae*'da %60 ve *Enterobacter aerogene*'de %100 olarak ifade edildi. XDR *E. coli*'de %50 ve *K. pneumoniae*'de %20 oranında ifade edilen kolistin direnci de aynı derecede endişe vericiydi. XDR *Enterobacteriaceae*'ye karşı olumlu aktiviteleri olan mono antibiyotikler arasında kolistin, tigesiklin ve meropenem bulunmaktaydı. TZP; CAZ ve CRO ile birleştirildiğinde XDR *E. coli* ve *K. pneumoniae* için sinerji gözlendi. AMC; CAZ veya GN ile birleştirildiğinde hiçbir sinerji gözlenmedi.
Sonuç: Bu çalışma, BSBH arasında XDR *Enterobacteriaceae* insidansını göstermiştir ve ESBL ve AmpC β-laktamazın ortak üreticileri de dahil olmak üzere XDR *Enterobacteriaceae*'ye bağlı enfeksiyonlar için yararlı bir tedavi kombinasyonu olarak TZP artı CAZ veya CRO'yu önermiştir.
Anahtar Kelimeler: Aşırı derecede ilaç direnci, çoklu ilaç direnci, *Enterobacteriaceae*, bağışıklığı baskılanmış hastalar, ESBL, AmpC, karbapenemaz, Katsina, Nijerya

Introduction

In addition to hygiene and strict infection control protocol adherence, antibiotics have been an important therapeutic tool for a wide variety of illnesses that are caused by bacteria. Regrettably, the use of these antibiotics has been accompanied by the rapid emergence of resistant strains that makes the treatment of common infections difficult or impossible[1,2].

The emergence of multi-drug-resistant (MDR) and extensively drug-resistant (XDR) bacteria among the members of the *Enterobacteriaceae* family is a major health problem that requires urgent attention since many of these bacteria are normal flora of the human gut and are easily transmissible to other human, animals, and the environment[3,4]. Easy acquisition and dissemination of these extremely resistant bacteria in hospitals, especially where infection control protocols are inadequate, has made the management of hospitalized patients more difficult. Of high concern are immunocompromised persons (ICPs), e.g., HIV infected individuals, the children, elderly, pregnant women, the malnourished, and those on steroids and other immunosuppressive drugs who are more vulnerable to resistant infectious agents due to their low immunity and long hospital stay[5,6].

The variability in the resistance pattern of pathogens that are isolated from different categories of ICPs, locally and regionally, requires an urgent review of the currently used empirical treatments in hospitals to manage ICPs who are infected with XDR bacteria to ensure the availability of appropriate treatments for their management[7].

Combining antibiotic therapy for XDR *Enterobacteriaceae* may become necessary due to the potential severity of infections they caused and the high risk of resistance selection that will ensue due to the use of monotherapies, especially among ICPs[8,9]. Several studies have reported different resistant mechanisms among different bacteria in Nigerian hospitals and have examined *in vitro* interactions between various antibiotics (e.g., β-lactams, colistin and polymyxin B, fosfomycin, aminoglycosides, and quinolones)[10-14]. However, no study has checked the presence of XDR *Enterobacteriaceae* members from ICPs and no clear recommendations were available as to the antibiotic combinations against XDR *Enterobacteria*, especially in ICPs. This study mainly aimed to determine the incidence of XDR among the members of *Enterobacteriaceae* that are isolated from hospitalized ICPs and immune-competent health care workers (IC-HCWs), screen them for β-lactamase production, and check the effectiveness of some antibiotics alone and in combination for XDR bacteria treatment.

Materials and Methods

Study Area

The study was conducted at the Federal Medical Center (FMC), Katsina, located in the capital city of Katsina State. The hospital is a 500-bed space tertiary hospital, which became operational in 1998 and provides tertiary health care services to the citizenry of the state and the neighboring countries. It is a referral facility, well-equipped and adequately staffed, rendering 24-h tertiary level medical services to a population of approximately 7,831,319 people[15].

Study Design

This prospective study was conducted from April to October 2018.

Sample Types

A total of 400 samples were proportionately collected from:

1. Hospitalized ICPs in FMC, Katsina, from the following wards: medical (male and female), surgical] (male, female, orthopedic, and pediatric), special care baby unit (SCBU), the antenatal ward, and the intensive care unit (ICU),

2. Healthy IC-HCWs,

3. Healthy immune-competent hospital administrative staff (IC-admin).

Sample Collections

Urine, wound swabs, sputum, stool, and catheter samples were appropriately obtained from the ICPs. Hand swabs were collected from the IC-HCWs and admin staff.

All specimens were collected and transported according to standard methods[16]. All patients and other participants provided written informed consent before participating in the study. The consent was obtained from the parents or guidance for children.

Inclusion and Exclusion Criteria

1. Clinical samples from identified admitted ICPs in the selected departments/wards, as well as healthy IC-HCWs and admin staff (who serve as controls) in the facility.

2. Samples with the pure growth of members of the *Enterobacteriaceae* family were included in the study.

Meanwhile, isolates other than members of *Enterobacteriaceae* were excluded from the study.

Ethical Consideration

Study approval was granted vide-FMCNA REC.REG.NO03/082012, dated March 12, 2018, by the Ethical Research Committee of FMC, Katsina, to conduct the study in the facility. Consent forms were also used to highlight the research purpose and procedures to the study participants, client's parents, or guardians as the case may be.

Bacterial Isolation and Identification

The culture media employed in this study include MacConkey agar, blood agar, chocolate agar, cysteine-lactose-electrolyte deficient agar, Salmonella-Shigella agar, and Mueller-Hinton agar (MHA), which were all prepared following the respective manufacturer instructions.

The samples were aseptically cultured on appropriate media and incubated at 37 °C for 24 h. Pure cultures of the bacterial isolates were then macroscopically and microscopically examined for their cultural morphology according to standard laboratory procedures. The respective bacterial colonies were stained by Gram's technique and biochemical tests, and indole, citrate utilization, urease production, and sugar fermentation were conducted for further identification. The isolates were then identified by comparing their characteristics with those of known taxa[16].

Screening for XDR Bacteria: Antibiotic Susceptibility Testing

The following categories of antibiotics (Oxoid, UK) were employed: amoxicillin (20 µg), amoxicillin/clavulanic acid (AMC) (20/10 µg), piperacillin/tazobactam (TZP) (100/10 µg), aztreonam (30 µg), tetracycline (30 µg), meropenem (10 µg), ciprofloxacin (5 µg), cefuroxime (30 µg), ceftriaxone (CRO) (30 µg), cefoxitin (30 µg), gentamicin (GN) (10 µg), tigecycline (15 µg), and colistin (10 µg). The selection was based on recommendations of the British Society for Antimicrobial Chemotherapy/Healthcare Infection Society/British Infection Association Joint Working Party on Treatment of infections caused by MDR Gram-negative bacteria[17]. Whereupon, an isolate was considered XDR when they are resistant to all the antimicrobial agents, but two or less and are considered MDR when they are resistant to at least three or more classes of antibiotics. Additionally, isolate that resist all the tested antimicrobial agents are considered pan drug-resistance (PDR). Antibiotic susceptibility testing was determined using the disc diffusion method on MHA according to the Clinical Laboratory Standards Institute (CLSI) guidelines[18].

Screening of the XDR Bacteria for β-lactamase Production

Single-drug resistance *Enterobacteriaceae* were phenotypically screened for extended-spectrum β-lactamases (ESBL) production according to the CLSI protocol using AMC and CRO and ceftazidime (CAZ) discs. The XDR isolates were further screened for AmpC β-lactamase production by co-inoculating a standard inoculum suspension of *E. coli* ATCC 25922 and test organism (XDR bacteria) on MHA as described elsewhere[19,20].

Moreover, XDR isolates were screened for carbapenemase production according to the CLSI 2018 guidelines using a Modified Hodges Test technique employing meropenem and imipenem (Oxoid, UK) antibiotic discs[18].

The metallo β-lactamase (MBL) producers among the XDR bacteria were screened using meropenem-ethylene diamine tetraacetic acid (EDTA) combined disk synergy test[19,21]. A control disc containing EDTA alone was used to determine EDTA activity to ensure that it does not cause false-positive results by inhibiting the test isolates.

Antibiotic Combinations

1. Checkerboard Assay

The minimum inhibitory concentrations (MICs) of the selected antibiotics (TZP, CRO, CAZ, AMC, and GN) against the 15 XDR *Enterobacteriaceae* isolates were first determined according to a standard procedure described by the CLSI in 2018. The tests were performed using a broth micro-dilution method on a 96-well plate in duplicates, and the plates were incubated at 37 °C for 24 h.

Using the MICs of the obtained selected antibiotics, a checkerboard assay was then designed to determine their fractional inhibitory concentration index (FICIs) in combinations against the XDR isolates. The test was also performed on a 96-well plate according to the CLSI guidelines[18] and as described in the literature[8,9]. Two-fold serial dilutions of TZP were placed in horizontal rows of the microtiter plate and were subsequently diluted vertically by 2-fold serial dilutions of CRO, CAZ, and GN.

The FIC was calculated based on the formula:

FIC of a particular antibiotic=MIC of the antibiotic in combination with other antibiotics divide by the MIC of the antibiotic alone. The FICIs were defined as the FIC of the two antibiotics used. For this study, the combining effect of CAZ and TZP against the XDR isolates were interpreted as follows: synergy, FICI ≤0.5; indifference, 0.5<FICI>4.0; and antagonism, FICI ≥4.0.

2. Antibiotic Disc Combination Test

The antibiotic synergy was further checked using a disc diffusion method prepared in-house. Stock solutions of CAZ, CRO, and GN were prepared from standard individual antibiotics according to the CLSI guidelines[18,19]. From there, serial dilutions were made to give their final respective concentrations of 30 µg for CAZ and CRO, as well as 10 µg for GN, per disc each in a total volume of 4 ml for 200 plain discs. The TZP discs were placed into three sterile labeled Petri dishes TZP+CRO, TZP+CAZ, and TZP+GN. The discs, which were adequately spaced to avoid overlap, were impregnated with appropriate volume at 20 µl (0.02 ml) of the diluted solutions of CAZ (30 µg), CRO (30 µg), and GN (10 µg). Likewise, AMC (20/10 µg, Oxoid) discs were as well separately impregnated as similarly done for TZP combinations. The prepared antibiotics were dried and placed in appropriately labeled containers.

Antibiotic susceptibility testing, using the combined antibiotic discs, was then determined by employing the disc diffusion technique on MHA according to the CLSI guidelines[18] and interpreted as sensitive (S), intermediate (I), or resistance (R).

Data Analysis

The results were presented in frequency distribution tables.

Statistical Analysis

Statistical analysis was performed using Minitab (version 16) software at a significance level of $p<0.05$.

Results

From the 400 obtained clinical samples of urine, stool, sputum, and wound swab from ICPs, as well as hand swabs from IC-HCWs, 68 members of the family *Enterobacteriaceae* were isolated, including *Escherichia coli (*41), *Klebsiella pneumoniae* (15), *Enterobacter aerogenes* (5), *Proteus mirabilis* (5), *Shigella* sp. (1), and *Morganella morganii* (1), with *E. coli* (60.3%) and *K. pneumoniae* (22.1%) as the most prevalent (Table 1).

Out of the 68 isolated *Enterobacteriaceae* members, 15 (22.1%) exhibited XDR status by being resistant to all but 1 or 2 classes of tested antimicrobial agents. Further, 72% of isolates from ICPs are MDR, but none exhibit PDR status. Contrastingly, none of the healthy IC-HCWs harbors XDR bacteria, but alarmingly, 5.9% of isolates from them were MDR (Table 1). Furthermore, the XDR isolates distribution across various sites of isolation in the hospital revealed that the female medical wards have the highest number of 4 (26.7%) XDR isolates followed by the pediatric medical/SCBU with 3 (20%) isolates and pediatric surgical ward with 2 (13.3%) XDR isolates, while others had

Table 1. Prevalence and resistance profile of *Enterobacteriaceae* isolated from ICPs and IC-HCWs/IC-ADMIN

Isolate type	Total no. isolated (%)	ICPs		IC-HCWs		IC-ADMIN	
		MDR	XDR	MDR	XDR	MDR	XDR
E. coli	41 (60.3)	30 (44.1)	8 (11.8)	3 (4.4)	0	0	0
K. pneumoniae	15 (22.1)	10 (14.7)	5 (7.4)	0	0	0	0
E. aerogenes	5 (7.4)	3 (4.4)	1 (1.5)	1 (1.5)	0	0	0
P. mirabilis	5 (7.4)	4 (5.9)	1 (1.5)	0	0	0	0
Shigella sp.	1 (1.5)	1 (1.5)	0 (0)	0	0	0	0
M. morganii	1 (1.5)	1 (1.5)	0 (0)	0	0	0	0
Total	68 (100)	49 (72.1)	15 (22.1)	4 (5.9)	0 (0)	0 (0)	0 (0)

ICPs: Immunocompromised patients, MDR: Multi-drug-resistant, XDR: Extensively drug-resistant, IC-HCWs: Immune-competent health care workers

1 (6.7%) each (Table 2). Among the XDR bacteria, *E. coli* was the most predominantly isolated (8/53.3%) followed by *K. pneumoniae* (5/33.3%), *E. aerogenes* (1/6.7%), and *P. mirabilis* (1/6.7%).

The distribution of XDR isolates, based on length of hospital stay and the nature of infection/conditions, which includes urological (with or without urinary catheters), surgical wounds (with subtypes), severe malnutrition, and pulmonary tuberculosis, was shown in Table 3. Approximately half (53.3%) of the patients that had XDR bacteria isolated had stayed for a period between 0 and 9 days in the hospital to receive treatment, whereas 33.3% stayed for 10-19 days and 13.3% (including patients with amputated limbs) had the longest stay of 20-29 days.

Table 4 shows the resistance profile of all XDR isolates, of which 100% expressed resistance to GN, TZP, aztreonam, CRO, amoxicillin, AMC, tetracycline, and ciprofloxacin. Additionally, *E. aerogenes* and *P. mirabilis* similarly expressed 100% resistance to cefoxitin and tigecycline.

The XDR isolates had variable resistance to meropenem, with *E. coli* having 37.5%, 60% in *K. pneumoniae*, and 100% in *E. aerogenes* isolate. The resistance to colistin was expressed by 50% of the *E. coli*, whereas 20% of the *K. pneumoniae*. Additionally, 75% resistance was recorded for *E. coli* and 60% by *K. pneumoniae* against tigecycline. Furthermore, 85% resistance against cefoxitin was recorded by *E. coli* followed by *K. pneumoniae* with 80% resistance.

Table 5 shows the type of β-lactamase that the XDR bacteria produced from ICPs with an overall recorded prevalence rate of ESBL production been 53.3% and 13.3% for AmpC. The highest prevalence of ESBL production was recorded in *E. coli* (33.3%), followed by *K. pneumoniae* (13.3%), with *P. mirabilis* (6.7%) as the last. Equally alarming is the co-production of ESBL and AmpC by 25% (n=2) of *E. coli* isolates. Additionally, none of the four isolates produced carbapenemase and MBL.

Table 6 shows the MIC values for TZP, AMC, CAZ, CRO, and GN on each of the XDR isolates. The MICs were outside the ranges published by the CLSI in 2019. MICs of the antibiotics against XDR bacteria that co-produce AmpC and ESBL were similar. Interestingly, *K. pneumoniae* isolated from AE/ICU, which does not produce ESBL, AmpC, and carbapenemase, showed the highest MIC for all antibiotics. The mean FICIs values determined by checkerboard assay are shown in Table 7. Synergy was observed for XDR *E. coli* and *K. pneumoniae* when TZP was combined with CAZ and CRO. No synergy was observed when AMC was combined with either CAZ or GN. The interaction

Table 2. Distribution of XDR isolates per site of isolation

Site of isolation	*E. coli*	*K. pneumoniae*	*E. aerogenes*	*P. mirabilis*	Total (%)
Male Medical	1	0	0	0	1 (6.7)
Female Medical	1	2	1	0	4 (26.7)
Male Surgical	0	0	0	1	1 (6.7)
Female Surgical	1	0	0	0	1 (6.7)
Ped. Surgical	2	0	0	0	2 (13.3)
ANC/ANW/GYNAE	1	0	0	0	1 (6.7)
Orthop. Ward	1	0	0	0	1 (6.7)
PMW/SCBU	1	2	0	0	3 (20)
AE/ICU	0	1	0	0	1 (6.7)
Total	8	5	1	1	15 (100)

Orthop. Ward: Orthopedic ward, ANC/ANW/GYNAE: Antenatal care/antenatal ward/gynecology wards, PMW/SCBU: Pediatric medical ward/special care baby units, AE/ICU: Accident and emergency/intensive care ward, XDR: Extensively drug-resistant

Table 3. Hospital stay of patients with XDR *Enterobacteriaceae* infections

Hospital stay (days)	Urological		DFT	Surgical			SAM	PTB	Total
	CAT	NO CAT		ORTH/AMP	S/WOUND	AC/BRU			
0-9	2 (13)	2 (13)	0 (0)	0 (0)	1 (7)	1 (7)	1 (7)	1 (7)	8 (53)
10-19	3 (20)	0 (0)	1 (7)	0 (0)	1 (7)	0 (0)	0 (0)	0 (0)	5 (33)
20-29	0	0	0	2 (13)	0 (0)	0 (0)	0 (0)	0 (0)	2 (14)
Total	5	2	1	2	2	1	1	1	15

CAT: With a catheter, NO CAT: No catheter, DFT: Diabetic foot, ORTH/AMP: Orthopedic amputation, S/WOUND: Surgical wound, AC/BRU: Accidental bruises, SAM: Severe acute malnutrition, PTB: Pulmonary tuberculosis, XDR: Extensively drug-resistant

between TZP and AMC with CAZ, CRO, and GN was indifferent against *E. aerogenes* and *P. mirabilis*.

Using the disc diffusion method, an increased zone of inhibition was observed in 4 XDR *E. coli* and 3 *K. pneumoniae*, when TZP and CAZ/CRO were combined (Table 8). Combining GN and TZP or AMC did not improve the susceptibility in all the isolates. *E. aerogenes* (n=1) and *P. mirabilis* (n=1) had no enhancements in the zone of inhibition with all the combinations except for a slight zone enhancement in CAZ and TZP combination against only *P. mirabilis* isolate.

Discussion

This study was conducted on ICPs, specifically those with chronic diseases, such as diabetes mellitus, renal failure, or the acquired immunodeficiency syndrome, surgical cases, pregnancy, and elderly that possess a high risk of acquiring antibiotic-resistant opportunistic pathogens most especially members of the family *Enterobacteriaceae*. The isolation of XDR bacteria among these categories of patients may dampen the hope of getting well. The analyzed samples from ICPs and IC-HCWs yielded isolation of 68 members of *Enterobacteriaceae* (*E. coli*, *K. pneumoniae*, *E. aerogenes*, *P. mirabilis*, *Shigella* species, and *M. morganii*). *E. coli* (60.3%) and *K. pneumoniae* (22.1%) were the most prevalent. Their presence was not surprising since ICPs are prone to various microorganisms during hospitalization. Invasive devices, such as catheters and ventilators, used on some patients could be a source of pathogens as previously reported[14,22,23].

The clinicians in the studied health facility use β-lactam antibiotics (CRO, CAZ, and cefuroxime), fluoroquinolones (ciprofloxacin), aminoglycosides (GN), and macrolides (azithromycin) in large volumes to treat patients. The widespread use of such antimicrobials for therapy or prophylaxis could be the major determinant of resistance that might have eventually resulted in the emergence and spread of XDR bacteria in health care settings[24,25]. However, last-resort antibiotics (colistin, tigecycline, and meropenem) showed slight but favorable activities against the XDR *Enterobacteriaceae* isolates. Non-frequent use due to high costs and toxicity could be the reason behind the low to moderate resistance exhibited by the isolates[8,26,27]. Of the 68 members of the *Enterobacteriaceae* isolates, 15 (22.1%) were XDR bacteria, which is lower than 65% as recorded by Hasanin et al.[28] in Egypt, as well as that studies

Table 4. Percentage of resistance of the XDR *Enterobacteriaceae* to different antibiotics

Antibiotics	*E. coli* (n=8)	*K. pneumoniae* (n=5)	*E. aerogenes* (n=1)	*P. mirabilis* (n=1)
CN	100	100	100	100
TZP	100	100	100	100
MEM	37.5	60	100	0
ATM	100	100	100	100
CXM	100	100	100	100
CRO	100	100	100	100
FOX	87.5	80	100	100
AML	100	100	100	100
AMC	100	100	100	100
TG	75	60	100	100
TE	100	100	100	100
CIP	100	100	100	100
CT	50	20	0	0

GN: Gentamicin, TZP: Piperacillin/tazobactam, MEM: Meropenem, ATM: Aztreonam, CMX: Cefuroxime, CRO: Ceftriaxone, FOX: Cefoxitin, AML: Amoxycillin, AMC: Amoxycillin/clavulanic acid, TG: Tigecycline, TE: Tetracycline, CIP: Ciprofloxacin, CT: Colistin, XDR: Extensively drug-resistant

Table 5. Percentage prevalence of ß-lactamase-producing XDR *Enterobacteriaceae*

Type of isolate (n)	ESBL (%)	AmpC (%)	Carbapenemase (%)	MBL (%)
E. coli (8)	5 (33.3)	2 (13.3)	0 (0)	0 (0)
K. pneumoniae (5)	2 (13.3)	0 (0)	0 (0)	0 (0)
E. aerogenes (1)	0 (0)	0 (0)	0 (0)	0 (0)
P. mirabilis (1)	1 (6.7)	0 (0)	0 (0)	0 (0)
Total (15)	8 (53.3)	2 (13.3)	0 (0)	0 (0)

ESBL: Extended-spectrum β-lactamases, MBL: Metallo β-lactamase

conducted across India[29,30]. However, our finding is comparably higher than 8.1%, and 13.8% XDR bacteria prevalence rates are recorded in other studies conducted in India[31,32].

Although no XDR bacteria was detected among the healthy IC-HCWs in the facility, the detection of 5.9% of MDR bacteria from their hands was also alarming and could serve as a

Table 6. *In vitro* activity of piperacillin-tazobactam/amoxicillin-clavulanic, ceftazidime cefuroxime, and gentamicin against XDR *Enterobacteriaceae*

Type of isolate	Source of isolates	Resistance phenotype	MIC (µg/ml)				
			TZP	AMC	CAZ	CRO	GN
E. coli	Male medical	ESBL, AmpC	≥128	≥32	≥16	≥32	16
E. coli	Female medical	ESBL	64	≥32	≥16	16	16
E. coli	Female surgical	ESBL	≥128	≥32	≥16	≥32	≥16
E. coli	Pediatric surgical	ESBL, AmpC	≥128	≥32	≥16	≥32	8
E. coli	Pediatric surgical	ESBL	64	≥32	8	≥32	8
E. coli	ANC/ANW/GYNAE	-	64	16	8	≥32	4
E. coli	Orthopedic ward		≥128	≥32	≥16	16	8
E. coli	PMW/SCBU		64	≥32	≥16	8	4
K. pneumoniae	Female surgical	ESBL	≥128	≥32	8	16	8
K. pneumoniae	Female surgical	-	64	≥31	≥16	≥32	≥16
K. pneumoniae	PMW/SCBU	ESBL	≥128	≥32	8	16	8
K. pneumoniae	PMW/SCBU	-	64	≥32	≥16	16	8
K. pneumoniae	AE/ICU	-	≥128	≥32	≥16	≥32	16
E. aerogenes	Female medical	-	≥128	≥32	8	16	8
P. mirabilis	Male medical	ESBL	64	≥32	≥16	16	8

CRO: Ceftriaxone, CAZ: Ceftazidime, XDR: Extensively drug-resistant, AMC: Amoxycillin/clavulanic acid, GN: Gentamicin, TZP: Piperacillin/tazobactam, ESBL: Extended-spectrum β-lactamases, ANC/ANW/GYNAE: Antenatal care/antenatal ward/gynecology wards, PMW/SCBU: Pediatric medical ward/special care baby units, AE/ICU: Accident and emergency/intensive care ward

Table 7. Mean FICI values of piperacillin-tazobactam/amoxicillin-clavulanic in combination with ceftazidime cefuroxime, and gentamicin

Type of isolate	Source of isolates	Resistance phenotype	FICI (µg/ml)					
			TZP+CAZ	TZP+CRO	TZP+GN	AMC+CAZ	AMC+CRO	AMC+GN
E. coli	Male medical	ESBL, AmpC	0.5	0.25	0.5	0.5	1	0.5
E. coli	Female medical	ESBL	0.25	0.25	0.5	1	2	1
E. coli	Female surgical	ESBL	0.5	0.5	0.25	0.25	0.5	1
E. coli	Pediatric surgical	ESBL, AmPC	1	0.25	0.5	1	0.5	1
E. coli	Pediatric surgical	ESBL	0.25	0.25	0.5	0.25	0.25	2
E. coli	ANC/ANW/GYNAE	-	0.25	0.25	0.5	0.25	1	0.5
E. coli	Orthopedic ward		0.25	0.25	1	1	2	1
E. coli	PMW/SCBU		0.5	0.25	1	0.25	0.5	2
K. pneumoniae	Female surgical	ESBL	0.25	0.25	2	0.25	0.5	0.5
K. pneumoniae	Female surgical	-	0.25	0.25	2	1	2	0.5
K. pneumoniae	PMW/SCBU	ESBL	0.5	1	1	2	1	1
K. pneumoniae	PMW/SCBU	-	1	0.5	0.5	0.25	1	1
K. pneumoniae	AE/ICU	-	0.25	0.25	0.5	1	0.5	1
E. aerogenes	Female medical	-	2	1	1	1	1	2
P. mirabilis	Male medical	ESBL	2	2	1	2	1	1

CRO: Ceftriaxone, CAZ: Ceftazidime, XDR: Extensively drug-resistant, AMC: Amoxycillin/clavulanic acid, TZP: Piperacillin/tazobactam, ESBL: Extended-spectrum β-lactamases, ANC/ANW/GYNAE: Antenatal care/antenatal ward/gynecology wards, PMW/SCBU: Pediatric medical ward/special care baby units, AE/ICU: Accident and emergency/intensive care ward, GN: Gentamicin

reservoir for cross-contamination to other patients, especially when infection control protocols are not well-practiced. A much higher percentage of MDR bacteria (18.4%) was earlier reported from the hands of the medical staff of the Yaoundé University Teaching Hospital in Cameroon[33].

The distribution of pathogen that causes infections, particularly antimicrobial-resistant, changes with time and varies among hospitals and locations in the same hospital[7,34,35]. This is also reflected in this study since the XDR bacteria distribution pattern varies across various units in the hospital, with the female medical ward having the highest number (4/26.7%) followed by the pediatric surgical and special care units. The use of invasive devices, excessive use of antibiotics, and longer stay in such wards could be the reason behind higher XDR bacteria isolation. Accordingly, frequent isolation of XDR and MDR bacteria is particularly evident in several European countries, such as Spain, Germany, and France, and has been associated with the contamination of hospital environments, invasive devices, and patients with complex treatment[36].

Further, patients who had catheters, surgical wounds, malnutrition, as well as pulmonary tuberculosis, had long periods of hospital stay. Prolonged stay of patients in the admission or ICU has been indicated for more antibiotic use, which will ultimately increase bacterial resistance to antibiotics[7,37]. The practice of prolonged pre- and post-operative antibiotic prophylaxis/treatment in orthopedic surgery to prevent/treat surgical site infections could have resulted in the development of XDR by bacteria that are isolated from patients who stayed longer in admission after orthopedic amputation surgery.

The pattern of antibiotic resistance expressed by the isolated XDR bacteria from the ICPs was quite disturbing since all the isolates resist almost all the available antibiotics in the facility. β-lactam antibiotics, as well as ciprofloxacin and GN, as the most predominantly prescribed antibiotics in treating bacterial infections in the facility, might have exerted higher selective pressure for the emergence of XDR *Enterobacteriaceae*. The association between increased rates of antimicrobial use and resistance has been documented for nosocomial infections, as well as for resistant community-acquired infections in Nigeria[10,38,39]. The high frequency of XDR and MDR bacteria among both ICPs and IC-HCWs might be a reflection of inappropriate use/misuse of antimicrobials, lack of laboratory diagnostic tests, and unavailability of guidelines for antibiotic selection. Expired antibiotics, self-medication, counterfeit drugs, and inadequate hospital infection control measures can as well promote the development of resistance in clinical isolates[16,40]. Increasing resistance to meropenem, colistin, and tigecycline is quite disturbing despite inadequate documented evidence for their widespread use in the health care facility probably due to their high costs, thereby narrowing down the treatment options for ICPs.

To further study the level of resistance, we analyzed the MIC values of five antibiotics against the XDR isolates. This is highly necessary to commence empirical therapy and predict and manage possible treatment failures[7]. The MIC result shows that almost all the XDR isolates show high MIC. The increased prevalence of high MIC was seen in all the extensively resistant *K. pneumoniae* and *E. coli*. However, previous studies in a

Table 8. Zones of inhibition (mm) and antibiotic susceptibility pçrofile of XDR isolates to a combination of antibiotics

Type of isolates	Resistance phenotype	TZP+CAZ	TZP+CRO	TZP+GN	AMC+CAZ	AMC+CRO	AMC+GN
E. coli	ESBL, AmpC	17 (I)	23 (S)	0 (R)	9 (R)	0 (R)	0 (R)
E. coli	ESBL	24 (S)	21 (S)	0 (R)	0 (R)	0 (R)	0 (R)
E. coli	ESBL	9 (R)	24 (S)	18 (I)	17 (I)	0 (R)	0 (R)
E. coli	ESBL, AmpC	0 (R)	22 (S)	10 (R)	9 (R)	14 (I)	9 (R)
E. coli	ESBL	22 (S)	24 (S)	10 (R)	24 (S)	12 (I)	0 (R)
E. coli	-	26 (S)	24 (S)	0 (R)	22 (S)	0 (R)	14 (I)
E. coli		24 (S)	24 (S)	0 (R)	0 (R)	0 (R)	0 (R)
E. coli		13 (R)	24 (S)	8 (R)	23 (S)	10 (R)	0 (R)
K. pneumoniae	ESBL	26 (S)	24 (S)	0 (R)	25 (S)	0 (R)	0 (R)
K. pneumoniae	-	25 (S)	24 (S)	0 (R)	0 (R)	8 (R)	0 (R)
K. pneumoniae	ESBL	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)
K. pneumoniae	-	10 (R)	0 (R)	0 (R)	12 (R)	0 (R)	0 (R)
K. pneumoniae	-	22 (S)	20 (S)	0 (R)	0 (R)	0 (R)	0 (R)
E. aerogenes	-	0 (R)	9 (R)	1	0 (R)	0 (R)	0 (R)
P. mirabilis	ESBL	0 (R)	0 (R)	1	0 (R)	8 (R)	0 (R)

S: Sensitive, R: Resistance, I: Intermediate, CRO: Ceftriaxone, CAZ: Ceftazidime, XDR: Extensively drug-resistant, AMC: Amoxycillin/clavulanic acid, TZP: Piperacillin/tazobactam, ESBL: Extended-spectrum β-lactamases, GN: Gentamicin

tertiary hospital in a neighboring state of Kano and other places have indicated carbapenem and colistin resistance among *Enterobacteriaceae*[39,41].

The overall prevalence of 53.3% ESBL producers among the XDR isolates in this study was much higher than the 15.0% recorded seven years before this current study in Kano[42], as well as the findings of Ogefere et al.[43] who earlier reported 44.3% prevalence in North Central region of Nigeria. Similarly, the prevalence of AmpC production among XDR isolates from ICPs is slightly higher than that in previous studies. A study conducted in some hospitals in Kano recorded variable AmpC productions of 10%[42] and 11.9%[44]. Co-production of AmpC and ESBL by 2 *E. coli* indicates a higher potential for resistance. The absence of carbapenemase in the isolates is surprising, which means that pathogens could attain XDR status without carbapenemase production. The resistance of some isolates that are not producing any type of carbapenemase equally supported the observation that Gram-negative bacilli could resist multiple antibiotics without producing β-lactamase. Although no PDR was recorded among the isolates, few studies have reported a 20% PDR prevalence among carbapenemase-producing *E. coli* isolated from healthcare facilities in Sokoto, Northwest Nigeria[45]. One study limitation is that noncarbapenemase production was not confirmed with a more specific polymerase chain reaction technique. The production of other hydrolyzing enzymes in conjunction with modifications in porin could be the reason for the high-level resistance to cephalosporin and carbapenem in this study[14,46].

Studies have indicated that treatment of infections caused by ESBL-, AmpC-, and carbapenemase-producing bacteria and other MDR/XDR bacteria will be more appropriate when various combination regimens are used[9,47-49]. However, there is a scarcity of information on how a combination of β-lactamase inhibitors and other antibiotics against XDR clones within the Nigerian setting could improve the treatment options. Considering the absence of carbapenemase-producing XDR isolates, we selectively investigated the combination of two β-lactamase inhibitors (TZP and AMC) and two oxyimino-cephalosporins (CAZ and CRO) and GN as a new effective treatment combination for ESBL-producing XDR isolates.

TZP, AMC, CAZ, CRO, and GN show discouraging activities when used to treat the XDR isolates by showing high MICs. A previous study revealed that mono-treatment of *Pseudomonas aeruginosa* clones with ceftolozane-tazobactam increases the development of resistance in the pathogens[36]. However, *in vitro* combination of TZP and CAZ or CRO in this study shows encouraging synergy for treating some strains of *E. coli that* produce ESBL and/or AmpC or co-produce the two. The effectiveness of combined antibiotics against strains of XDR *E. coli* and *K. pneumoniae* is promising since infections in ICPs are often caused by more than one resistant organism, thereby extending the antimicrobial spectrum that is necessary to eliminate stubborn bacteria. A combination of amikacin and CAZ, colistin, and meropenem was reported to synergistically improve the treatment option of XDR *Pseudomonas aeruginosa* [50].

The combination of CAZ, CRO, and GN with AMC shows no synergy. TZP+CAZ synergy was 87.5% against XDR *E. coli* strains. Similarly, TZP+CRO synergy was 75% and 60% against XDR *E. coli* and *K. pneumoniae* respectively. The combination of TZP and GN was indifferent against *K. pneumonia*, and the susceptibility of *E. aerogenes* and *P. mirabilis* did not improve with all the combinations except TZP+CAZ against the *P. mirabilis* isolate.

The excellent synergy in our study, which was achieved by combining TZP and any of CAZ and CRO, maybe due to the individual effect of β-lactams on several essential penicillin-binding proteins in the bacteria and tazobactam exhibits β-lactamase inhibition, which is also the basis for the previous combination of ceftolozane-tazobactam with meropenem against *P. aeruginosa* sequence type 175[36]. The confirmation of checkerboard assay that results with disc diffusion assay was a new approach to confirm the observed synergy.

Study limitations included our inability to confirm the presence or absence of β-lactamase production in the XDR isolates using molecular technique due to financial constraints. The absence of patients' antibiotic treatment history before their admission into the facility was unavailable to further substantiate the reason for XDR development. Finally, we were unable to test the immune status of patients in comparison with the immune-competent staff during the study.

Conclusion

In conclusion, this is the first study that (1) investigated the incidence of XDR in Nigeria and (2) investigated oxyimino-cephalosporin and GN in combination with TZP and AMC against XDR *Enterobacteriaceae* from IPCs. Our results demonstrate the presence of extensive drug-resistance among members of *Enterobacteriaceae* isolated from various categories of ICPs. Some XDR isolates are ESBLs and/or AmpC β-lactamase producers. Antibiotics with favorable activities against the XDR isolates include colistin, tigecycline, and meropenem, as well as combinations of TZP with either CRO or CAZ.

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Ethics

Ethics Committee Approval: Study approval was granted vide-FMCNA REC.REG.NO03/082012, dated March 12, 2018, by the Ethical Research Committee of Federal Medical Center, Katsina, to conduct the study in the facility.

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: I.Y., Design: I.Y., Data Collection or Processing: A.S.K., Analysis or Interpretation: A.S.K., U.A.D., Literature Search: A.S.K., Writing: U.A.D., I.Y.

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