Molecular screening of antibiotic-resistant determinants among multidrug-resistant clinical isolates of *Proteus mirabilis* from SouthWest Nigeria.

Olumuyiwa Samuel Alabi[1], Nuno Mendonça[2], Olufemi Ezekiel Adeleke[1], Gabriela Jorge da Silva[2,3]

1. Department of Pharmaceutical Microbiology, University of Ibadan, Nigeria, West Africa.
2. Center for Neurosciences and Cell Biology, University of Coimbra, Coimbra, Portugal
3. Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal

Abstract

Background: Globally, and particularly in developing countries, the menace of anti-microbial resistance is an accelerating problem. In Nigeria, increase in bacterial resistance has been phenotypically established but due to high cost, few molecular studies have been reported.

Objectives: This study screened for presence of transferable resistance genes and mobile genetic elements (MGEs) such as integron among multi-drug resistant (MDR) *P. mirabilis*.

Methods: A total of 108 *P. mirabilis* strains collected from five tertiary hospitals in SouthWest Nigeria were subjected to antibiotic susceptibility study using disc-diffusion method. Transferable resistance genes and MGEs were amplified using Polymerase chain reaction (PCR) analysis and amplicons sequenced.

Results: Varied resistance was observed against all the antibiotics tested. About 56% of the isolates were MDR including those from 0-12 years old children. PCR analysis revealed the presence of *aac(6')-Ib* (33.3%), plasmid mediated quinolone resistance (PMQR) genes *[qnrA* (36.7%), *acc(6')-Ib-cr* (5%)], TEM (48.3%), CTX-M (6.7%) and integrons class 1 (58.3%) and class 2 (26.7%). Sequencing analysis revealed $bla_{\text{TEM-1}}$, $bla_{\text{CTX-M-15}}$ associated with IS*Ecp1* and eight different arrays of gene cassettes: *aadA1*, *aadA1-qacH, aadB-aadA2, aadA5, dfrA7, dfrA15, dfrA17, dfrA17-aadA5*.

Conclusion: Transferable resistance genes in association with MGEs are present in Nigerian *P. mirabilis* thus their potential in disseminating resistance.

Keywords: Multidrug resistance, resistance determinants, integrase, gene cassettes, *Proteus mirabilis*.

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Introduction

Anti-microbial drug resistance has become a global concern recognized by the World Health Organization[1]. *Proteus* species, common inhabitants of the soil and part of the normal flora of the enteric region of man and animals, have been found to cause opportunistic infections in several anatomical sites.[2,3] Among the various species of these genera, *Proteus mirabilis* is of medical importance and usually responsible for most of the common nosocomial opportunistic infections such as urinary tract infections, wounds, ear and other infections.[3] Their possession of a number of virulence factors including antibiotic resistance genes, have placed the organism on the list of medically important nosocomial agents.[4,5,6,7,8] The micro-organism has been implicated in several nosocomial infection outbreaks and community acquired infections in different parts of the world, including Nigeria.[9,10,11,12] Among other antibiotics used in the treatment of nosocomial infections in Nigeria, the commonest classes of antibiotics usually employed for life threatening cases are the third generation cephalosporins, fluoroquinolones and aminoglycosides. However, several authors have reported resistance of *Enterobacteriaceae* including *Proteus mirabilis* to these classes of antibiotics.[13,14,15,16]

Corresponding author:
Gabriela Jorge da Silva,
Faculty of Pharmacy and Center
for Neurosciences and Cell Biology,
University of Coimbra, Health Sciences
Campus, Azinhaga de Santa Comba,
3000-548 Coimbra, Portugal.
Tel: + 351 239488460.
Email: gjsilva@ci.uc.pt;
silva.gj@gmail.com

Resistance to third generation cephalosporins has been attributed to the presence of certain resistance determinants called extended-spectrum beta-lactamase enzymes, ESBLs.[17] Several variants of these enzymes which were believed to have evolved from the wild-type beta-lactamases (TEM-1 and SHV-1) via mutation at one or more point in the gene coding for their amino acid sequence exist and are spread among bacterial isolates worldwide.[17] Another widely spread ESBL group are the CTX-M-type discovered in the 1980s in Munich, Germany from clinical isolate of *Escherichia coli* and currently five phylogroups exist.[18] They are known to have high hydrolytic property against cefotaxime.[18]

Resistance to fluoroquinolones was initially thought to be majorly due to chromosomal mutation involving gyrA and topoisomerase genes until plasmid mediated quinolone resistance genes (PMQR) such as the *qnr, qepA* and *aac(6')-Ib-cr* was later reported in 1998.[19] Since then PMQR genes have been described in several bacterial isolates particularly members of the *Enterobacteriaceae* family worldwide.[13,15,20,21,22] The *qnr* genes are known to produce proteins (QNR proteins) that protect the quinolone targets from inhibition. Studies have shown that they produce a low level resistance, but facilitate a higher level resistance in association with chromosomal mutations in bacteria harbouring them.[23,24,25] The aac(6')-Ib-cr, which encode a variant of aminoglycoside transferase that confers reduced susceptibility to ciprofloxacin and norfloxacin by N-acetylation of their piperazinyl amine, causes an increase in their MIC two to four fold[26] and *qepA* gene, which encodes quinolone-specific efflux pump[27] initiate decreased susceptibility to quinolones by up to 64-fold.[28] Resistance to aminoglycosides such as gentamicin, amikacin and tobramycin, have been attributed to the aminoglycoside-modifying enzymes. Among the different classes of these aminoglycoside-modifying enzyme that have been reported, the one encoded by the *aac(6')-Ib* gene is the most commonly isolated in many clinical isolates particularly among the *Enterobacteriaceae* worldwide.[13,15]

Common resistance determinant genes that have been detected among isolates belonging to the *Enterobacteriaceae* family in Nigeria are ESBLs (TEM, CTX-M, SHV and OXA), AmpC Beta-Lactamase (CMY, DHA and ACT), plasmid-mediated quinolone resistance (*qnrA, qnrB, qnrD, aac(6')-Ib-cr* and *qepA*) and aminoglycoside resistance (*aac(6')-Ib*) genes.[15,29] Most of these resistant genes are acquired from other bacterial isolates through mobile genetic elements such as integrons.

Integrons are composed of three key elements: a gene encoding an integrase, a primary recombination site, and a promoter that directs the transcription of captured genes.[30] Integrase is involved in capturing of resistance genes and incorporating them by site-specific recombination within the integrons conserved segments.[30]

The aim of this study was to screen for the presence of common transferable resistance genes and mobile genetic elements such as integron and their associated gene cassettes among MDR clinical isolates of *P. mirabilis* from SouthWest Nigeria.

Methods

Clinical bacterial isolates

A total of 108 non-duplicated clinical isolates of *P. mirabilis* were randomly collected during a 12 months period (January to December, 2011) from Microbiology units of five selected tertiary hospitals in SouthWest Nigeria, namely University Teaching Hospital (UCH) Ibadan (n = 43), Olabisi Onabanjo University Teaching Hospital (OOUTH) Sagamu (n = 10), Federal Medical Centre (FMC) Abeokuta (n = 13), Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife (n = 28) and Lagos state University Teaching Hospital (LUTH) Lagos (n = 14). The isolates were obtained from different clinical samples (wound, ear, eye, vaginal swabs, urine, sputum, peritoneal effluents, finger abscesses, pus and stool). They were identified by using Gram stain, MacConkey and Chocolate agar, swarming activity and confirmed with Microbact 12E Identification kit (Oxoid Ltd. Basingstoke, Hants, UK). Information on the patient's data such as age, sex and specimen types was also recorded.

Antibiotic susceptibility test

The clinical isolates were subjected to antibiotic susceptibility testing against twelve antibiotics (Oxoid Ltd. Basingstoke, Hants, UK): amoxicillin (10μg), amoxicillin-clavulanic acid (20/10μg), cefoxitin (30μg), ceftazidime (30μg), cefotaxime (30μg), aztreonam (30μg), imipenem (10μg), gentamicin (10μg), amikacin (30μg), nalidixic acid (30μg), ciprofloxacin (5μg), and trimethoprim-sulfamethoxazole (25μg), using the disc-diffusion method. Minimum Inhibitory Concentrations (MICs) of selected

antibiotics (cefotaxime, ceftazidime, amoxicillin-clavulanic acid, ciprofloxacin and gentamicin) against selected MDR strains was done by broth micro-dilution using microtiter plates to further ascertain their level of resistance. Interpretation was done by comparing results with Clinical and Laboratory Standards Institute guidelines.[31]

Amplification of common resistance genes

Clinical isolates that showed resistance to at least three classes of antibiotics were regarded as MDR isolates[32] and were selected for amplification of resistance genes using polymerase chain reaction (PCR) technique.

Beta-Lactamase gene detection

MDR strains that showed resistance to third generation cephalosporins (cefotaxime and ceftazidime), aztreonam but were susceptible to cefoxitin were screened by simplex PCR for bla_{TEM}, bla_{SHV} and bla_{CTX-M} gene.[33,34,35] The CTX-M-positive strains were screened by simplex PCR for commonly associated insertion sequences (*ISEcp1, IS26* and *IS903*) using them and bla_{CTX-M} primer as forward and reverse respectively.[36] Those MDR isolates that showed resistance to the third generation cephalosporins, aztreonam and cefoxitin but were susceptible to imipenem were screened for common variants of AmpC (bla_{CMY}, bla_{DHA-1} bla_{FOX}) and bla_{OXA} that are usually found in *Enterobacteriaceae* using a multiplex PCR.[37]

Detection of genes encoding plasmid mediated quinolone resistance (PMQR) and aminoglycoside-modifying enzyme (AAC).

MDR isolates with reduced susceptibility or resistance to quinolones and aminoglycosides were screened for the presence of *qnrA, qnrB, qnrS, qepA* and *aac(6')-Ib* genes using multiplex PCR.[21] The *aac(6')-Ib* amplicons were further digested with BstF5I (New England Biolabs, Ipswich, MA) and electrophoresed on 1% agarose gel to identify the *aac(6')-Ib-cr* which lacks the BstF5I restriction site present in the wild-type *aac(6')-Ib* gene.[38]

Detection of classes 1, 2 and 3 Integrons and associated gene cassettes

All the MDR isolates were screened for the presence of classes 1, 2 and 3 integron-associated integrase genes using specific primers.[39] The variable region of the integrase positive strains were then amplified by PCR using specific primers to know if they carry gene cassettes.[39]

Purification and sequencing of amplified PCR products

The resulting PCR amplification products of the detected beta-lactamase genes and the amplified variable region of the integrons were purified using *GF-1* PCR cleanup kit according to the manufacturer's guidelines (Qiagen, Izasa, Portugal) and then sequenced in both forward and reverse nucleotide chains (Stabvida, Portugal) to identity the genes.

Conjugation assay for ESBL positive strains

The conjugation assay was performed using a modified method described by Kim et al. (2004).[12] The ESBL positive donor strains were conjugated with the standard strain *E. coli* J53 (resistant to sodium azide) as a recipient cell. MacConkey agar containing 4 μg/mL of cefotaxime and 200 μg/mL of sodium azide was used as the selection medium. The growth of the sodium azide resistant *E. coli* J53 in the selection plate was interpreted as an indication of an ESBL embedded plasmid transfer. The transconjugants were confirmed by determining the antimicrobial susceptibility profile and PCR amplification.

Mutagenic treatment of the isolates harbouring ESBL genes

Isolates harbouring ESBL genes were subjected to R-plasmid curing experiment using the modified method described by Adeleke et al. (2002).[40] Briefly, the overnight culture of each ESBL-positive *P. mirabilis* strains was exposed to 200 and 100 μg/ml of ethidium bromide and incubated for 24 hours at 37^0C. Pure colonies of the ethidium bromide treated and untreated isolates were first selected on MacConkey agar plates and then on Nutrient agar, after which they were then diluted to 0.5 MacFarland standard for susceptibility testing against selected antibiotics (cefotaxime, ceftazidime, aztreonam, ciprofloxacin, gentamicin and trimethoprim-sulphamethoxazole) by disc-diffusion method, and zones of inhibition interpreted by comparing with the CLSI guideline.[31]

Results

Source of bacterial isolates and antimicrobial susceptibility testing

Out of 108 clinical isolates of *P. mirabilis*, 47 (43.5%) were from infected wounds, 24 (22.2%) from infected ears, 23 (21.3%) from urine of patients suffering from urinary

tract infections, 4 (3.7%) from infected vaginal swabs, 3 (2.8%) from stool of diarrheic patients, 2 (1.9%) from infected eye exudate, 1 (0.9%) from peritoneal effluent of patient with kidney disease, 1 (0.9%) from sputum of patient with infected respiratory tract, and 1 (0,9%) from pus of finger abscess. Twenty nine (26.9%) of the isolates were obtained from children within the ages of 0 to 17 years, 67 (62%) from patients within the ages 18 to 59 years and 12 (11.1%) from patients above 60 years.

The result of the anti-microbial susceptibility test showed that 80 (74.1%) of the isolates were resistant to trimethoprim-sulfamethoxazole, 70 (64.8%) to amoxicillin, 58 (53.7%) to nalidixic acid, 36 (33.3%) to cefotaxime, 29 (26.9%) to gentamicin, 26 (24.1%) to ceftazidime, 17 (15.7%) to amoxicillin-clavulanic acid, 16 (14.8%) to aztreonam, 15 (13.9%) to ciprofloxacin,7 (6.5%) to cefoxitin, 5 (4.6%) to amikacin and 2 (1.9%) to imipenem.

Sixty (55.6%) of the isolates were classified as MDR since they were resistant to at least three classes of antibiotics. Twelve (20%) of these MDR isolates were from children within 0 – 12 years of age suffering from various infections such as otitis media (50%), conjunctivitis (8.3%), burn wounds (25%), abscess (8.3%) and UTI (8.3%). The MICs of the selected antibiotics against some of the MDR isolates are presented in Table 1.

Table 1: Antibiotic susceptibility profile and resistant genes detected in multidrug resistant clinical isolates of *Proteus mirabilis*.

Isolate ID	Clinical Sample	Patient' Demography		Antibiotic MIC (µg/mL)						Resistant genes detected						Gene cassette arrays							
		Age (Yrs)	Sex	NA	CIP	GN	AMC	CTX	CAZ	qnrA	aac(6')-Ib-cr	$bla_{CTX-M-15}$	bla_{TEM-1}	aac(6')-Ib	aadA1	aadA1-qacH	aadA2-aadB	aadA5	aadA5-	dfrA7	dfrA15	dfrA17
Pm 02	ECS	32	F	>256	4	64	8	64	>256	+	-	-	+	+	-	-	-	-	-	-	-	+
Pm 03	Urine	50	F	>256	4	64	8	64	>256	+	-	-	+	+	-	-	-	-	+	-	-	-
Pm 04	Urine	21	M	>256	4	64	0.5	64	256	+	-	-	+	+	-	-	-	-	-	-	-	-
Pm 07	Ear pus	44	F	>256	<0.25	32	0.5	0.5	1	-	-	-	-	-	-	-	-	+	-	-	-	-
Pm 09	Ear swabs	28	M	>256	1	64	0.5	32	256	+	-	-	+	+	-	-	-	-	-	-	-	+
Pm 10	Urine	26	F	>256	4	64	0.5	256	>256	+	-	-	+	+	-	-	-	-	-	-	-	+
Pm 12	Wound swabs	51	M	>256	2	64	0.5	32	256	+	-	-	+	+	-	-	-	-	-	-	+	-
Pm 17	Wound swabs	14M	M	>256	2	64	>256	64	>256	+	-	-	-	+	-	-	-	-	-	-	-	+
Pm 20	Urine	28	M	>256	4	64	0.5	256	>256	+	-	-	+	+	-	-	-	-	-	-	-	-
Pm 24	Ear swabs	112D	F	>256	2	64	0.5	64	256	+	-	-	+	+	-	-	-	-	-	-	-	-
Pm 26	PE	22	F	>256	2	64	0.5	256	>256	+	-	-	+	+	-	-	-	-	-	-	-	-
Pm 27	ECS	26	F	>256	1	64	0.5	64	256	+	-	-	+	+	-	-	-		-	-	-	-
Pm 30	Ear swabs	35	M	>256	4	64	2	64	256	+	-	-	+	+	-	-	-	-	-	-	-	+
Pm 31	Ear swabs	2½	F	>256	4	64	64	32	256	+	-	-	+	+	-	-	-	-	-	-	-	+
Pm 32	Urine	30	F	>256	4	64	2	32	256	+	-	-	+	+	-	-	-	-	-	-	-	-
Pm 33	Urine	47	F	>256	4	64	2	32	256	+	-	-	+	+	-	-	-	-	-	-	-	-
Pm 34	Urine	28	F	>256	4	64	0.5	32	256	+	-	-	+	+	-	-	-	-	-	-	-	-
Pm 44	Wound swab	29	M	>256	16	128	8	128	>256	-	+	+	+	-	-	-	-	-	+	-	-	-
Pm 47	Ear swabs	10	M	>256	2	64	2	32	64	+	-	-	+	+	-	-	-	-	-	-	-	-
Pm 49	Ear swab	11	M	>256	0.5	16	256	0.5	1	-	-	-	-	-	+	-	-	-	-	-	-	-
Pm 68	Wound swab	50	F	>256	4	128	16	32	128	+	-	-	+	+	-	-	-	-	-	-	-	+
Pm 73	Wound swab	35	M	>256	1	32	8	128	128	-	-	+	-	-	-	-	-	-	+	-	-	-
Pm 74	Wound swab	33	M	>256	0.5	32	4	0.5	8	-	-	-	-	-	-	-	-	-	-	+	-	-
Pm 79	Wound swab	60	F	>256	0.25	32	4	0.5	1	-	-	-	-	-	-	+	-	-	-	-	-	-
Pm 92	Urine	38	M	>256	2	64	4	64	128	+	-	-	-	-	-	-	-	-	-	-	-	-
Pm 93	Wound swabs	55	F	>256	0.5	128	4	64	64	-	-	-	-	+	-	-	-	-	-	-	-	-
Pm 94	Wound swabs	7	M	>256	4	32	64	64	64	+	-	-	-	-	-	-	-	-	-	-	-	-
Pm 100	Wound swabs	36	F	>256	16	256	16	128	128	+	+	+	+	-	-	-	-	-	-	-	-	-
Pm 107	Wound swabs	6	M	>256	2	64	256	128	128	+	-	+	+	-	-	-	+	-	-	-	-	-
Pm 114	Wound swabs	55	F	>256	2	128	8	32	32	-	-	-	+	+	-	-	-	-	-	-	-	-
Pm 116	Wound swabs	37	M	>256	16	256	256	32	16	-	+	-	+	-	-	-	-	-	-	-	-	-

Key: ID – Identity, M – Male, F – Female, ECS – Endo-cervical swabs, PE – Peritoneal effluent, NA – Nalidixic acid, CIP – Ciprofloxacin, GN – Gentamicin, AMC – Amoxicillin-clavulanic acid, CTX – cefotaxime, CAZ – ceftazidime, MICs – Minimum Inhibitory Concentrations, + – presence of gene, - – absence of gene, Yrs – Years, Pm – *Proteus mirabilis*.

Antimicrobial resistance genes

Amplification and sequencing of the beta-lactamase genes from the MDR strains resistant to cefotaxime or/and ceftazidime revealed that 29 (48.3%) harbored bla_{TEM-1} and 4 (6.7%) harboured $bla_{CTX-M-15}$. The $bla_{SHV,}$ bla_{C-MY}, bla_{OXA}, bla_{DHA-1} and bla_{FOX} genes were not found. Two of the $bla_{CTX-M-15}$ genes were associated with *ISEcp1*. One of the $bla_{CTX-M-15}$ genes was detected from an MDR isolate collected from a six years old male child with an infected burn wound (Table 1).

Twenty (33.3%) of the MDR isolates carried the *aac(6')-Ib* gene, including two isolated from a 2½ and 10 year old female and male patients, respectively, both suffering from ear infections (Table 1). The *aac(6')-Ib-cr* and qnrA genes, associated with quinolone resistance, were present in 3 (5%) and 22 (36.7%) of the MDR isolates, respectively, while none was positive for *qepA*.

All (100%) MDR isolates harboured integrase genes, either *IntI1*, *IntI2* or both, among which 96.7% harboured IntI1, 53.3% *IntI2*, 51.7% carried both *IntI1* and *IntI2* while none harboured *IntI3*. Amplification of the integrons variable region produced positive amplicons in 76.7%, which included 58.3% and 26.7% of the *IntI1* and *IntI2* positive strains respectively. Nine of the children within the ages 0 and 12 years were infected with integron-positive MDR strains. Sequencing analysis revealed eight different gene cassette arrays (*aadA1, aadA1-qacH, aadB-aadA2, aadA5, dfrA7, dfrA15, dfrA17* and *dfrA17-aadA5*) spread among the integron positive MDR isolates. The gene cassette array *dfrA17-aadA5* (47.1%) was the most common among the MDR isolates.

Conjugation and curing assays

The conjugation experiment performed with four CTX-M-15 strains did not yield any noticeable growth of transconjugants in the selection plates. Moreover, the curing experiment did not alter the susceptibility profile of the CTX-M-15 positive isolates to the cephalosporins and aztreonam significantly but rather increased the susceptibility of the isolates to the effect of the sulphamethoxazole-trimethoprim, gentamicin and to ciprofloxacin, as shown in Table 2.

Table 2: Antibiotic susceptibility profiles of the mutagen treated and untreated CTX-M-15 positive clinical isolates of MDR *P. mirabilis*

Isolate ID	Antibiotic Susceptibility Profile					
	CTX	CAZ	AT	CIP	GN	SXT
	Zones of growth inhibitions (mm)					
Untreated Clinical isolates of *P. mirabilis*						
Pm 044	R	R	S	R	R	R
Pm 073	R	R	S	I	R	R
Pm 100	R	R	S	R	R	R
Pm 107	R	R	S	R	R	R
Clinical isolates of *P. mirabilis* treated with 100µg/mL Ethidium bromide						
Pm 044	R	R	S	S	S	S
Pm 073	R	R	S	I	S	S
Pm 100	R	R	S	S	S	S
Pm 107	R	R	S	R	S	S
Clinical isolates of *P. mirabilis* treated with 200µg/mL Ethidium bromide						
Pm 044	R	R	S	S	S	S
Pm 073	R	R	S	R	S	S
Pm 100	R	R	S	S	S	S
Pm 107	R	R	S	R	S	S

Pm – *Proteus mirabilis*, S – Sensitive, I = Intermediate, R = Resistant, CTX = Cefotaxime, CAZ = Ceftazidime, ATM = Aztreonam, CIP = Ciprofloxacin, GN = Gentamicin, SXT = Sulphamethoxazole-trimethoprim.

All the primers used in the amplification of the genes detected are presented in table 3.

Table 3: List of Primers used in this study

Target gene	Primers	Primer Sequence (5'- 3')	Amplicon size (bp)	References
OXA-1 variants	OXA-F	GGCACCAGATTCAACTTTCAAG	564	Caroline *et al*., 2010
	OXA-R	GACCCCAAGTTTCCTGTAAGTG		
DHA-1 variants	DHA-1-F	TGATGGCACAGCAGGATATTC	997	Caroline *et al*., 2010
	DHA-1-R	GCTTTGACTCTTTCGGTATTCG		
Multiplex *CMY* (2-7,12-18,21-23)	CMY-F	CGAAGAGGCAATGACCAGAC	538	Caroline *et al*., 2010
	CMY-R	ACGGACAGGGTTAGGATAGY		
Multiplex *FOX*	FOX-F	CTACAGTGCGGGTGGTTT	162	Caroline *et al*., 2010
	FOX-R	CTATTTGCGGCCAGGTGA		
bla_{CTX-M}	CTX-M-F	TTTGCGATGTGCAGTACCAGTAA	543	Edelstein *et al.*, 2003
	CTX-M-R	CGATATCGTTGGTGGTGCCATA		
bla_{TEM}	TEM-F	TACGATACGGGAGGGCTTAC	716	Belaaouaj *et al*., 1994
	TEM-R	TTCCTGTTTTTGCTCACCCA		
bla_{SHV}	SHV-F	TCAGCGAAAAACACCTTG	471	M'Zali *et al*., 1996
	SH-R	TCCCGCAGATAAATCACCA		
qnrA	qnrA-F	ATT TCT CAC GCC AGG ATT TG	516	Kim *et al*., 2009
	qnrA-R	GAT CGG CAA AGG TTA GGT CA		
qnrB	qnrB-F	GAT CGT GAA AGC CAG AAA GG	476	Kim *et al*., 2009
	qnrB-R	ATG AGC AAC GAT GCC TGG TA		
qnrS	qnrS-F	GCA AGT TCA TTG AAC AGG GT	428	Kim *et al*., 2009
	qnrS-R	TCT AAA CCG TCG AGT TCG GCG		
aac(6')-Ib	aacIb-F	TTG CGA TGC TCT ATG AGT GGC TA	482	Kim *et al*., 2009
	aacIb-R	CTC GAA TGC CTG GCG TGT TT		
qepA	qepA-F	AAC TGC TTG AGC CCG TAG AT	596	Kim *et al*., 2009
	qepA-R	GTC TAC GCC ATG GAC CTC AC		
IS*Ecp1*	IS*Ecp1*	AAAAATGATTGAAAGGTGGT	Variable	Eckert *et al*., 2004
IS*26*	IS26	AGCGGTAAATCGTGGAGTGA	Variable	Eckert *et al*., 2004
IS*903*	IS903	CGGTTGTAATCTGTTGTCCA	Variable	Eckert *et al*., 2004
IntI1	Int 1-F	CAG TGG ACA TAA GCC TGT TC	160	Dillon *et al*., 2005
	Int 1-R	CCC GAG GCA TAG ACT GTA		
IntI2	Int 2-F	CAC GCA TAT GCG ACA AAA AGG T	788	Dillon *et al*., 2005
	Int 2-R	GTA GCA AAC GAG TGA CGA AAT G		
IntI3	Int 3-F	GCC TCC GGC AGC GAC TTT CAG	979	Dillon *et al*., 2005
	Int 3-R	ACG GAT CTG CCA AAC CTG ACT		
Class 1 array	hep58	TCA TGG CTT GTT ATG ACT GT	Varied	Dillon *et al*., 2005
	hep59	GTA GGG CTT ATT ATG CAC GC		
Class 2 array	hep74	CGG GAT CCC GGA CGG CAT GCA CGA TTT GTA	Varied	Dillon *et al*., 2005
	hep51	GAT GCC ATC GCA AGT ACG AG		

Discussion

P. mirabilis is becoming a common opportunistic pathogen particularly within the Nigerian hospital setting.[41] *P. mirabilis* is often associated with urinary tract infections especially among patients with long-term indwelling catheters or under frequent antibiotic therapy.[42,43] However, in this study, high occurrence in wound infections (43.5%) was found compared to urinary tract infections (21.3%) but a similar percentage occurred in ear infections (22%). Other studies performed in Nigeria, though with a lower number of isolates, also reported a higher isolation of *P. mirabilis* in wounds than in urine.[3,15] The reason for the observed increased cases of wounds infected with *P. mirabilis* in previous and current studies cannot be explained but calls for serious caution in infection control program in these hospitals. Though the majority of the patients (62%) were between the age of 18 and 59 years, about 30% of the isolates were recovered from infected children less than 17 years (including newborn and children that were a few months old). This should be a worrisome development to healthcare professionals, highlighting that control of nosocomial infection must be drastically improved in Nigerian tertiary hospitals.

The high frequency of MDR phenotype observed among the *P. mirabilis* isolates in this study (55.6%) confirms earlier reports that MDR is increasing in Nigeria among members of the *Enterobacteriaceae* family.[15] It is a concern to see that 20% of the MDR isolates detected in this study are from children between the ages 0 and 12 years. This may not be surprising since various reports mention that self-medication of children is rampant among nursing mothers, who usually take antibiotics irrationally even during breastfeeding.[44,45,46,47]

A low prevalence of $bla_{\text{CTX-M-15}}$ was found, (6.7%) contrary to the 27.3% prevalence of bla_{SHV} and $bla_{\text{CTX-M-15}}$ reported by Ogbolu et al.[15] among 11 clinical *P. mirabilis* isolates from SouthWest Nigeria. Although $bla_{\text{CTX-M-15}}$ has been previously described in clinical *P. mirabilis* isolates in Southwest Nigeria,1[5] this study reported IS*Ecp1* associated $bla_{\text{CTX-M-15}}$ usually involved in the transposition of the gene and its dissemination.[48] The association of $bla_{\text{CTX-M-15}}$ with mobile genetic elements such as insertion sequences and conjugative plasmids usually explain its worldwide dissemination. However, the conjugation assays performed in this study with all the CTX-M-15 positive strains did not produce any transconjugants suggesting that it is not likely to be associated with conjugative plasmid. Furthermore, the R-plasmid curing experiments with ethidium bromide against these strains retained the resistance profile conferred by the $bla_{\text{CTX-M-15}}$, this still suggest a non-plasmid based gene. Although $bla_{\text{CTX-M-15}}$ was already found within a chromosomal location associated with IS*Ecp1* in *P. mirabilis* strains isolated in Tunisia[49] and Korea,[50] the two phenotypic experiment done in this study are inadequate to conclude a chromosomally based $bla_{\text{CTX-M-15}}$. A molecular analysis, which was not the objective of this study, is required to finally confirm the actual genetic location of the $bla_{\text{CTX-M-15}}$ detected in this study.

Plasmid mediated quinolone resistance (PMQR) genes are known to confer resistance to nalidixic acid and to increase the minimum inhibitory concentrations of fluoroquinolones up to 32-fold or more.[26] In this study, the detection of *qnrA* and *aac(6')-Ib-cr* associated with the reduced susceptibility to fluoroquinolones showed that these genes are spreading among clinical isolates in Nigeria. Previously, these genes have been reported in some *Enterobacteriaceae* in Nigeria, together with the *qnrD* gene variant which was not detected in this study.[15]

The *aac(6')-Ib* gene is the most prevalent aminoglycoside modifying enzyme, conferring resistance to tobramycin, kanamycin and amikacin.[51] The gene was first identified in *Klebsiella pneumoniae* isolates in 1986 and since then several variants of this enzyme have been described.[26,51]

However, in this study, 3 (5%) of the MDR isolates harboured *aac(6')-Ib-cr*, a variant of the *aac(6')-Ib* gene.

Of relevance is the high prevalence of MDR *P. mirabilis* strains with class 1 and 2 integrase genes, *Intl1* and *Intl2*, and the occurrence of eight different gene cassette arrays (*aadA1, aadA1-qacH, aad*B-*aadA2, aadA5, dfrA7, dfrA15, dfrA17* and *dfrA17-aadA5*) within the variable region of the integrons in some of the isolates. The size of the amplicons (variable region) ranged from 300bp to 600bp depending on the type and number of gene cassettes present. This result shows the potential of these opportunistic micro-organisms to acquire resistance genes, which is a threat for under-resourced countries, where more potent antibiotics against MDR strains are usually expensive or inexistent.

Overall, accumulation of resistant determinants through the activities of mobile genetic elements is associated with the observed multidrug phenotype of *P. mirabilis*

clinical isolates from SouthWest Nigeria. Our findings showed that these strains have the tools to acquire resistance genes as much as possible. Therefore, strict surveillance of anti-microbial resistance, rational prescription of antibiotics and strict laws to be enforced against over-the-counter sales of antibiotics must be implemented in Nigeria, to reduce to a minimum antibiotic resistance cases in the country. The frequent isolation of *P. mirabilis* in infected wounds in tertiary hospitals in Nigeria suggests the urgent need to review the existing infection control measures in hospital settings and to educate the clinical staff on this scenario.

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Conflict of interest

None to declare.

Ethical approval

Not required.

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