DOI: 10.7860/JCDR/2018/30684.12123

Original Article

Microbiology Section

Erythromycin Resistance Determinants in Clinical Gram Positive Cocci Isolated from Nigerian Patients

DAVID OLUSOGA OGBOLU[1], OYEBODE ARMSTRONG TERRY ALLI[2], ADEOLU SUNDAY OLUREMI[3], CHRISTIANAH O ONIFADE[4]

ABSTRACT

Introduction: The emergence of erythromycin resistant Gram positive cocci in various parts of the world has become worrisome in clinical settings in recent times, however there is little or no information on the determinants of erythromycin resistance from Nigeria.

Aim: This study investigated the determinants of erythromycin resistance in clinical Gram positive cocci bacteria isolated from Nigerian patients.

Materials and Methods: Assembly of isolates of non-duplicate staphylococci from various clinical specimens from south western and northern hospitals of Nigeria were collected. While enterococci were obtained from our culture collection which were previously collected from healthy people from community. Characterisation of 25 staphylococci and enterococci each were done using standard microbiology procedures, susceptibility pattern to erythromycin and other panel of antimicrobial agents including Minimum Inhibitory Concentrations (MICs) to erythromycin was determined. Erythromycin resistance genes were amplified using Polymerase Chain Reaction (PCR).

Results: None of the strains had *ermA* and *mefA* but the strains showed heterogeneous possession of *ermB*, *ermC*, *msrA* and *msrB* genes in no particular pattern including multiple gene acquisition. MIC_{50} and MIC_{90} of staphylococci strains to erythromycin were 2 µg/mL and >64 µg/mL, respectively; wherein 7 (28%) were sensitive to erythromycin, while 11 (44%) of enterococci were sensitive to erythromycin with MIC_{50} and MIC_{90} of 1 and >64 µg/mL, respectively. One of the staphylococci isolates had inducible clindamycin resistance.

Conclusion: In conclusion, high level staphylococci and enterococci resistance was found to various antibiotics with limited therapeutic option. Ribosomal methylation and efflux are the main resistant determinants found in these isolates.

Keywords: Clindamycin, Efflux pump, Resistance determinants, Staphylococci

INTRODUCTION

Macrolides such as erythromycin constitute an important group of drugs because of their antimicrobial and immunomodulatory activities [1]. Erythromycin is a clinically important antibiotic which binds to a site in 23S rRNA on the large ribosomal subunit 50S close to the peptidyl transferase, near the entrance to the nascent peptide exit tunnel [2]. The resistance to antimicrobial agents is an increasingly global problem worldwide, especially among nosocomial pathogens. Resistance to macrolides such as erythromycin is prevalent among Gram positive cocci [3,4]. Erythromycin resistance can be due to target-site modification by an rRNA-methylating enzyme or by an efflux system. Target-site modification can be expressed either in a constitutive or inducible manner, resulting in co-resistance to Macrolide-Lincosamide-Streptogramin B (MLSB) antibiotics [5]. However, while bacterial resistance is continuously increasing as a result of antibiotic usage in human and veterinary medicine as well as in farms and in agriculture, the industrial antibiotic pipeline has progressively dried up [6]. On several occasions, bacteria isolated from patients are resistant to most of the antibiotics used in the hospital [7].

Staphylococci have become one of the most common causes of nosocomial infections with ability to develop resistance to antibiotics which were hitherto susceptible [8]. There have been several reported cases of erythromycin resistance in major Gram positive cocci pathogens worldwide; in a teaching hospital in Hatay, Turkey a total of 165 out of 298 isolates were resistant to erythromycin, and contained at least one of the erythromycin resistance genes; *ermA*, *ermB*, *ermC* and *msrA* [9], while erythromycin resistance in alpha- and beta-haemolytic streptococci was mediated through *erm* and/or *mef* genes in Hong Kong [10]. High levels of Gram positive cocci resistant to erythromycin has been reported in phenotypic studies carried out in different parts of Africa; in Johannesburg, South Africa 38% of the isolates were resistant to erythromycin [11], also, in Nigeria two separate phenotypic studies reported *S. aureus* isolates to be 30.7% intermediate and 21.2% resistant [12] and 28.57% resistant [13].

The mechanisms of resistance of erythromycin genes are well known elsewhere, but previous studies in Nigeria have only focused on phenotypic determination of erythromycin resistance [12,13], hence there is little or no information on the determinants of erythromycin resistance. Identifying the mechanisms of Gram positive cocci resistance to erythromycin will be of immense value in curbing and providing appropriate control measures for Gram positive cocci isolates. Therefore, the determinants of erythromycin resistance in clinical Gram positive cocci from Nigerian patients was investigated.

MATERIALS AND METHODS

Bacterial Isolates

A total of 50 isolates comprising 25 convenient non-duplicate assemblies of staphylococci which were collected for a period of three months between January and March 2015 in a cross-sectional/observational study and 25 enterococci from culture collection previously isolated from stool samples of apparently healthy members of Osogbo community in Osun State as a commensal flora who never visited hospital in the last three months. The staphylococci were from five different tertiary hospitals: University College Hospital, Ibadan; Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife; Ladoke Akintola University of Technology (LAUTECH) Teaching

Hospital, Osogbo; LAUTECH Teaching Hospital, Ogbomoso and Federal Medical Centre, Gusau. They were obtained from sick people in these hospitals from varying clinical sites such as urine, blood culture, wound, eye swab, ear swab, high vaginal swab and semen from inpatients and outpatients with varying diseases ranging from urinary tract infections, septicaemia, otitis media and conjunctivitis. The inclusion criteria include all staphylococci isolates from outpatients and inpatients and enterococci from stool samples of healthy members of the community. Also, non-staphylococci isolates from the hospitals including all isolates from intensive care unit and non-enterococci isolates from stool samples of healthy members were excluded from the study including their duplicate strains. Ethical approval was obtained for the study including informed consent from the participants. These isolates were identified using standard procedures as described in Medical Microbiology manual [14].

Antibiotic Susceptibility Testing

The antibiotic susceptibility patterns of all isolates to a panel of antibiotics namely; mupirocin (200 µg), tetracycline (30 µg), linezolid (30 µg), erythromycin (15 µg), gentamicin (30 µg), teicoplanin (30 µg), clindamycin (2 µg), cefoxitin (30 µg) were determined by the disc diffusion method in Mueller-Hinton agar (discs from Oxoid, UK). Staphylococci and enterococci isolates were considered sensitive (≥23 mm), intermediate (14-22 mm) and resistant (≤13 mm) for erythromycin based on the guidelines of Clinical Laboratory Standards Institute [15]. Erythromycin resistant isolates were selected further for MIC to erythromycin using microdilution method [15]. All runs included the control organism Oxford *S. aureus* NCTC 6571.

Inducible Clindamycin Resistance

Double disk diffusion (D-test) and microdilution tests were used to determine inducible clindamycin resistance [15]. Inocula of bacteria were prepared to 0.5 McFarland standards and sterile swab stick was used to streak the Mueller-Hinton agar. Erythromycin disc (15 µg) was placed 20 mm apart from a clindamycin disc (2 µg) on the surface of Mueller-Hinton plate and incubated at 37°C for 24 hour.

DNA Extraction and Amplification of Erythromycin Genes

DNA was extracted from 500 µL overnight Mueller-Hinton broth of each isolates of staphylococci and enterococci using lysostaphin and lysozyme to digest the cell wall as previously described by Alli O et al., [16]. Conventional PCR was used to detect genes encoding resistance to erythromycin (*ermA*, *ermB*, *ermC*, *mefA*, *msrA* and *msrB*) using a GeneAmp PCR system 9700 thermal Cycler (Applied Biosystems) [Table/Fig-1] [17,18]. Amplimers resulting from these PCR reactions were sequenced to confirm the identity and specific variant of each gene identified and sequences were aligned to known reference sequences using ClustalW [19].

Gene	Primer Sequence	Annealing Temperature (°C)	Product Size (bp)
ermA	ATGAACCAGAAAAACCCTAAA	52	732
	GGCTTAGTGAAACAATTTGTAAC		
ermB	GGCGGATGAACAAAAATATAAAATA	55	738
	GCGTTATTTCCTCCCGTTAAA		
ermC	GGCATGAACGAGAAAAATATAAA	46	735
	GCTAATATTGTTTAAATCGTCAAT		
mefA	AGTATCATTAATCACTAGTGC	50	367
	TTCTTCTGGTACAAAAGTGG		
msrA	CGATGAAGGAGGATTAAAATG	50	1737
	CATGAATAGATTGTCCTGTTAATT		
msrB	TATGATATCCATAATAATTATCCAATC	50	595
	AAGTTATATCATGAATAGATTATCCTATT		

[Table/Fig-1]: Primers used for the amplification of erythromycin resistance genes.

STATISTICAL ANALYSIS

Data were analysed using statistical package within the Epi-info software for Centre for Disease Control and Prevention, USA. Chi-square test was used to determine the association between various resistance genes and erythromycin resistance in *S. aureus* and CoNS. The p-value <0.05 was considered to be statistically significant.

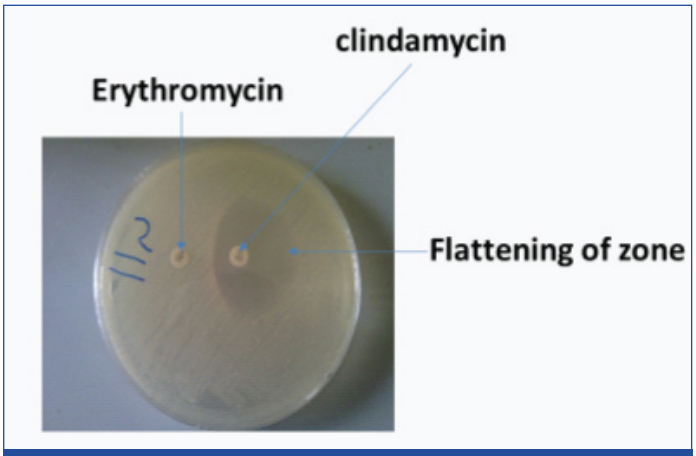
RESULTS

The susceptibility patterns of all the strains against a panel of antimicrobial agents including erythromycin are reported in [Table/Fig-2]. The antimicrobial susceptibility pattern revealed various degree of resistance to the antibiotics. Highest degree of antibiotic resistance to *S. aureus* was obtained for gentamicin 81.3%, this was followed by cefoxitin 56.3%, while CoNS had highest resistance of 88.9% to cefoxitin while gentamicin and clindamycin 44.4% each. The highest degree of antibiotic resistance to *Enterococcus* was recorded for tetracycline, 18/25 (72%) while linezolid was 100% sensitive (panel III) [Table/Fig-2]. A flattening of the zone of inhibition around the clindamycin disc proximal to the erythromycin disc was considered as a positive result. Only one of the staphylococcal isolates showed inducible clindamycin resistance [Table/Fig-3].

The MICs of the two species of staphylococci to erythromycin showed MIC_{50} and MIC_{90} of 2 and >64 µg/mL respectively while the MIC of enterococci strains against erythromycin showed MIC_{50} and MIC_{90} of 1 and >64 µg/mL respectively. In staphylococci 32% (8/25) had erythromycin resistance of MIC between 8 and >64 µg/mL while 40%

Antibiotic (µg/mL)	Resistant (%)	Intermediate (%)	Sensitive (%)
Panel I: 16 *Staphylococcus aureus*			
Mupirocin (200)	1 (6.3)	6 (37.5)	9 (56.2)
Tetracycline (30)	8 (50)	0 (0)	8 (50.0)
Linezolid (30)	2 (12.5)	0(0)	14 (87.5)
Erythromycin (15)	8 (50.0)	7 (43.8)	1 (6.2)
Gentamicin (30)	13 (81.2)	0 (0)	3 (18.8)
Teicoplanin (30)	1 (6.3)	2 (12.5)	13 (81.2)
Clindamycin (2)	8 (50)	0 (0)	8 (50.0)
Cefoxitin (30)	9 (56.2)	0(0)	7 (43.8)
Panel II: 9 CoNS			
Mupirocin (200)	0 (0)	3 (33.3)	6 (66.7)
Tetracycline (30)	3 (33.3)	2 (22.2)	4 (44.4)
Linezolid (30)	1 (11.1)	0 (0)	8 (88.9)
Erythromycin (15)	3 (33.3)	0 (0)	6 (66.7)
Gentamicin (30)	4 (44.4)	1 (11.1)	4 (44.4)
Teicoplanin (30)	0 (0)	0 (0)	9 (100)
Clindamycin (2)	4 (44.4)	1 (11.1)	4 (44.4)
Cefoxitin (30)	8 (88.9)	0 (0)	1 (11.1)
Panel III: 25 Enterococci			
Mupirocin (200)	NA	NA	NA
Tetracycline (30)	18 (72)	0 (0)	7 (28)
Linezolid (30)	0 (0)	0(0)	25 (100)
Erythromycin (15)	0 (0)	3 (12)	22 (88)
Gentamicin (30)	NA	NA	NA
Teicoplanin (30)	3 (12)	0 (0)	22 (88)
Clindamycin (2)	NA	NA	NA
Cefoxitin (30)	NA	NA	NA

[Table/Fig-2]: Antimicrobial disc susceptibility pattern of bacteria isolates.
NA: Not applicable; CoNS: Coagulase negative *staphylococcus*



[Table/Fig-3]: Inducible Clindamycin Resistance test: An inducible clindamycin resistance showing flattening of the zone of inhibition around the clindamycin disc proximal to the erythromycin disc.

Organism (No. of Strains)	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	Sensitive (%)	Range (µg/mL)
S. aureus (16)	2	>64	4 (25.0)	0.06-64
CoNS (9)	2	>64	3 (33.3)	0.06-64
Enterococci (25)	1	>64	11 (44.0)	0.06-64

[Table/Fig-4]: Minimum Inhibitory Concentration of 50 Gram positive cocci isolates to erythromycin.
CoNS: Coagulase negative staphylococcus

(10/25) showed intermediate MIC between 1 and 4 µg/mL with a total of 72% resistance including intermediate. Similarly, enterococci had 28% erythromycin resistance and intermediate each [Table/Fig-4].

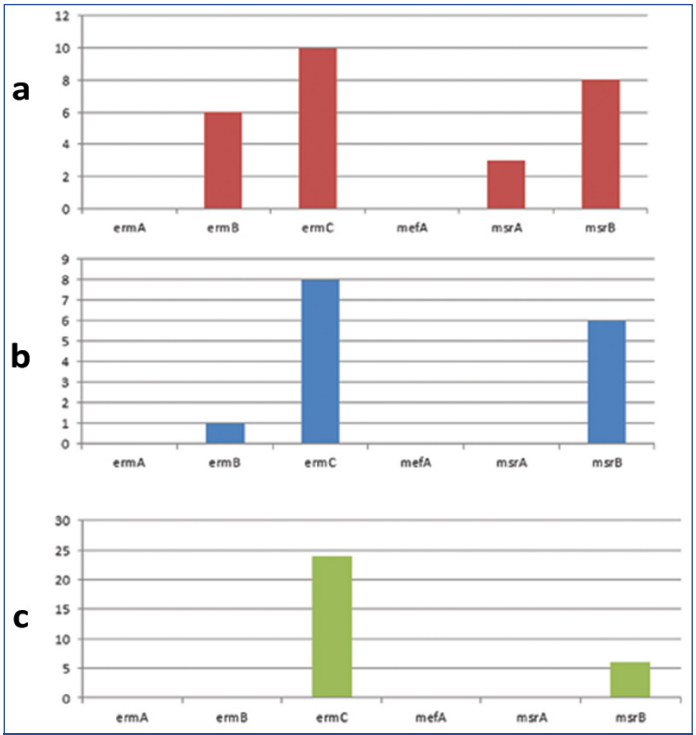
The genotypic properties of the isolates are shown in [Table/Fig-5]. The distribution of erythromycin resistance genes in each of the isolates is shown in [Table/Fig-6]. The *ermC* was the most prevalent resistant gene in *S. aureus*, 10/16 (62.5%) followed by *msrB*, 8/16 (50.0%), *msrA* was the least prevalent 3/16 (18.8%); these were found in both inpatient and outpatient [Table/Fig-6a]. The *ermC* and *msrB* were the most prevalent resistant genes in CoNS; 8/9 (88.9%) and 6/9 (66.7%) respectively, while *ermB* was the least prevalent 1/9 (11.1%) [Table/Fig-6b]. In enterococci strains, *ermC* was the most prevalent gene; 23/25 (92.0%) followed by *msrB* 6/25 (24.0%), while *ermA*, *ermB*, *msrA* and *mefA* were not found [Table/Fig-6c]. Six strains had *ermC* and *msrB*. It is noteworthy that *ermA* and *mefA* were consistently not amplified in all the three species and some isolates carried multiple genes of 2 to 4 [Table/Fig-5]. Statistically, there was no association between the presence of *ermB* gene and erythromycin resistance in *S. aureus* (χ^2=0.33, p=0.56), and same for CoNS (χ^2=0.141, p=0.71). Furthermore, no association was found between the presence of *ermC* and erythromycin resistance in *S. aureus* (χ^2=1.42, p=0.23), neither for CoNS (χ^2=0.14, p=0.71).

Similarly, the sources of the isolates showed that *FMC Gusau* had

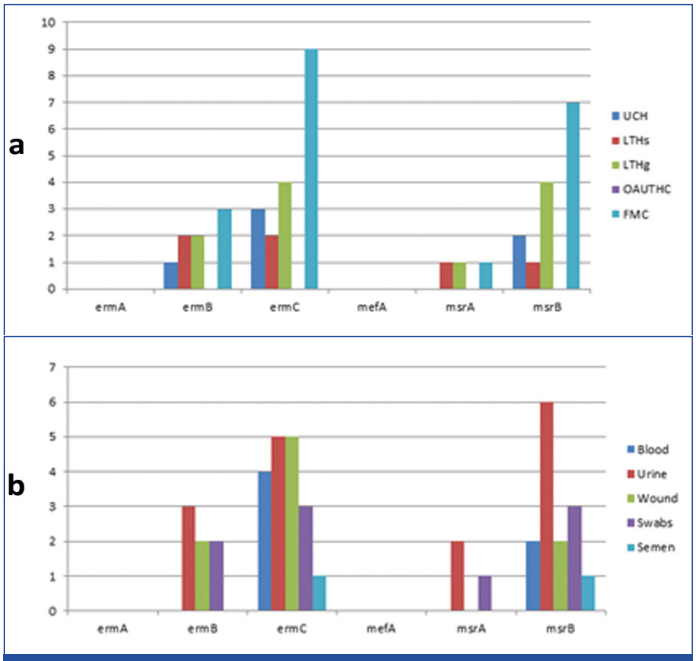
Lab No	Specimen	Source	In/Out patient	MIC (µg/mL) Erythromycin	Genes					
					ermA	*ermB*	*ermC*	*mefA*	*msrA*	*msrB*
Staphylococcus aureus										
S1	Blood	UCH	Inpatient	16	-	-	+	-	-	-
S2	Urine	LTH (Os)	Inpatient	0.06	-	+	+	-	+	+
S3	Urine	LTH (Og)	Inpatient	0.25	-	-	+	-	-	+
S4	Wound	UCH	Outpatient	4	-	-	+	-	-	+
S5	Ear swab	LTH (Os)	Inpatient	4	-	-	-	-	-	-
S7	Eye swab	LTH (Og)	Inpatient	2	-	+	+	-	+	+
S8	Urine	UCH	Inpatient	2	-	+	+	-	-	+
S10	Wound	FMC	Inpatient	0.5	-	+	+	-	-	-
S11	Wound	FMC	Inpatient	8	-	+	-	-	-	-
S12	Urine	FMC	Outpatient	8	-	+	-	-	-	-
S13	Urine	LTH (Og)	Inpatient	>64	-	-	-	-	-	+
S14	Wound	OAUTHC	Outpatient	1	-	-	-	-	-	-
S16	Urine	FMC	Outpatient	8	-	-	+	-	+	+
S21	Blood	LTH (Os)	Inpatient	0.5	-	-	+	-	-	-
S24	Wound	LTH (Os)	Inpatient	4	-	-	-	-	-	-
S25	Semen	FMC	Outpatient	1	-	-	+	-	-	+
Coagulase Negative *Staphylococcus*										
S6	Urine	LTH (Og)	Inpatient	0.5	-	-	-	-	-	-
S9	Blood	FMC	Inpatient	0.5	-	-	+	-	-	+
S15	Wound	FMC	Inpatient	>64	-	-	+	-	-	-
S17	Blood	FMC	Inpatient	2	-	-	+	-	-	+
S18	Wound	FMC	Inpatient	2	-	-	+	-	-	+
S19	Wound	LTH (Og)	Inpatient	4	-	-	+	-	-	-
S20	Ear swab	LTH (Og)	Outpatient	8	-	+	+	-	-	+
S22	HVS	FMC	Outpatient	>64	-	-	+	-	-	+
S23	Urine	FMC	Inpatient	0.25	-	-	+	-	-	+
Enterococcus spp										
E1	Stool	Community	NA	0.5	-	-	-	-	-	-
E2	Stool	Community	NA	64	-	-	+	-	-	+
E3	Stool	Community	NA	0.5	-	-	+	-	-	+
E4	Stool	Community	NA	1	-	-	+	-	-	+

E5	Stool	Community	NA	0.25	-	-	+	-	-	+
E6	Stool	Community	NA	4	-	-	-	-	-	-
E7	Stool	Community	NA	>64	-	-	+	-	-	+
E8	Stool	Community	NA	4	-	-	+	-	-	+
E9	Stool	Community	NA	>64	-	-	+	-	-	-
E10	Stool	Community	NA	2	-	-	+	-	-	-
E11	Stool	Community	NA	0.25	-	-	+	-	-	-
E12	Stool	Community	NA	0.125	-	-	+	-	-	-
E13	Stool	Community	NA	0.5	-	-	+	-	-	-
E14	Stool	Community	NA	1	-	-	+	-	-	-
E15	Stool	Community	NA	0.125	-	-	+	-	-	-
E16	Stool	Community	NA	16	-	-	+	-	-	-
E17	Stool	Community	NA	4	-	-	+	-	-	-
E18	Stool	Community	NA	0.5	-	-	+	-	-	-
E19	Stool	Community	NA	0.25	-	-	+	-	-	-
E20	Stool	Community	NA	8	-	-	+	-	-	-
E21	Stool	Community	NA	8	-	-	+	-	-	-
E22	Stool	Community	NA	2	-	-	+	-	-	-
E23	Stool	Community	NA	0.5	-	-	+	-	-	-
E24	Stool	Community	NA	8	-	-	+	-	-	-
E25	Stool	Community	NA	0.25	-	-	+	-	-	-

[Table/Fig-5]: Genotypic properties of Gram positive cocci isolates.
NA: Not Applicable; LTH (Os): Ladoke Akintola University Teaching Hospital; Osogbo; LTH (Og): Ladoke Akintola University Teaching Hospital, Ogbomoso; FMC: Federal Medical Centre; Gusau OAUTHC: Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife; UCH: University College Hospital, Ibadan; Community: healthy community members

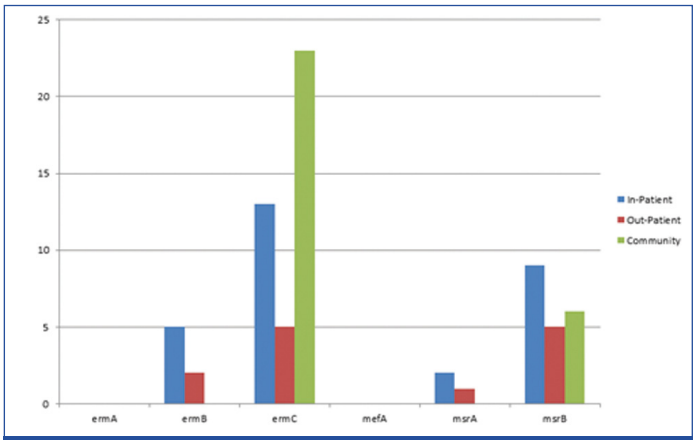


[Table/Fig-6]: Distribution of erythromycin resistance genes in; (a) S. aureus; (b) CoNS; (c) *Enterococcus spp*.



[Table/Fig-7]: Distribution of erythromycin genes according to the sources of the isolates.

the highest number of genes; *ermB*, *ermC* and *msrB* of 3 (12.0%), 7 (28.0%) and 9 (36.0%) respectively while Ladoke Akintola University of Technology Teaching Hospital Ogbomoso (LTH Ogbomoso) had 1 (4.0%) *ermB*, while *ermC* and *msrB* had 4 (16.0%) each. Isolates from Obafemi Awolowo University Teaching Hospital Complex (OAUTHC) had no resistant gene [Table/Fig-7], Also, inpatients had more frequency of the genes compared to outpatients, while community or carrier isolates which are essentially enterococci had 23/25 (92.0%) *ermC*, 6 (24.0%) *msrB* with no other genes present [Table/Fig-8].



[Table/Fig-8]: Distribution of erythromycin genes amongst the community, inpatients and outpatients.

DISCUSSION

The resistance level of *Staphylococcus* spp. has been on the increase as a result of widespread utilisation and uncontrolled use of erythromycin [20]. The MIC confirmed the results of disc diffusion that showed high level of erythromycin resistance; *S. aureus* was more resistant at 75% followed by CoNS 66.7%, all the isolates had MIC_{90} >64 µg/mL. There is possibility of intermediate susceptibility becoming full blown resistance if clinical use of erythromycin is not controlled. The presence of inducible clindamycin resistance in one of the staphylococci isolates indicates that inducible clindamycin resistance may be a major problem in the future if appropriate measures are not applied. Previous studies have shown a varying level of inducible resistance among the staphylococci [5,21].

Erythromycin resistance can be caused by several mechanisms, the predominant form being target modification mediated by one or more *erm* genes encoding a 23S rRNA methylase. Target site modification is mediated by the presence of *erm* genes; *ermA*, *ermB* and *ermC* [22]. The incidence may be influenced by geographical locations including variability from hospital to hospital, the source or origin of isolates, clinical samples or patient types and so on. The *ermC* was the most frequent gene, found in high number in all the isolates; enterococci had the highest followed by *S. aureus* and CoNS. Similarly, it was found more in inpatient than outpatient and this applies to other genes, overall more resistant genes were found in inpatients than outpatients. *ermC* has been regarded as the most widely disseminated and clinically important determinant of MLSB resistance in staphylococci [23]. A study conducted in south western part of Nigeria showed that 4 (two *S. epidermidis*, one *S. haemolyticus* and, *S. cohnii* each) out of five erythromycin resistant isolates possessed ermC genes, while *ermA* and *ermB* genes were absent [24]. In other countries such as Italy and Tunisia, *ermC* was also reported as the prevalent gene in clinical isolates of erythromycin resistant *S. epidermidis* [25,26]. One of the erythromycin resistant *S. haemolyticus* strain was found to possess the *msrA* gene which encoded an ATP-dependent efflux pump conferring resistance to 14- and 15-membered macrolides [7]. *ermA* was not found in both staphylococci and enterococci isolates, this is a sharp contrast to some previous studies where varying prevalence of ermA were found [27,28] while *ermB* was not found in enterococci it was found in staphylococci isolates (*S. aureus* and CoNS).

The resistance to erythromycin caused by the presence of macrolide efflux pumps in staphylococci (encoded by *msrA*, *msrB* or *mefA*) has also been documented [30]. The *msrA* was present only in *S. aureus* with a low frequency while *msrB* was found both in the staphylococci and enterococci isolates and was the most predominant gene after *ermC*. It was also observed that isolates which possess *msrA* also have *msrB*; the genes were found in different geographical locations and in different clinical sites. This study however revealed that carriage of *msrA* may not be connected with nosocomial acquisition as it was found in both inpatients and outpatients. Previous studies reported the prevalence of *msrA* to range from none to low [9,30]. The *mefA* gene was not found in both the staphylococci and enterococci isolates in present study.

All the erythromycin resistant isolates were positive for one or more of *ermB*, *ermC*, *msrA* or *msrB*. There was no association between the presence of erythromycin resistance genes, *ermB*; *ermC* and erythromycin resistance in *S. aureus* and CoNS. *FMC Gusau* had the highest frequency of *ermB*, *ermC* and *msrB*. The *ermB*, *ermC*, *msrA* and *msrB* genes may be the genes responsible for erythromycin resistance as found in this study. In general, major heterogeneity was detected in erythromycin resistance as regards the genotypes and the location of the hospitals. Overall, there is no particular pattern of MIC for the presence of *erm* or *msr* variants indicating there may be other mechanisms of resistance. A larger sample to determine the prevalence of these genes collected from different parts of the country may be necessary to establish their circulation in Nigeria and detailed molecular mechanisms will be of immense value to curbing the erythromycin resistance.

LIMITATION

This present study was limited because there was no grant hence, the use of small sample size and lack of detailed epidemiological study.

CONCLUSION

In conclusion, high level antibiotic resistance was found in both hospital and community isolates. The *ermC* and *msrB* representing ribosomal methylation and efflux respectively are the main resistant determinants with major genotypic heterogeneity in the staphylococci from the hospitals and enterococci from healthy community members.

ACKNOWLEDGEMENTS

The authors thank Dr. Mark A webber of Antimicrobial Research Group, Institute of Microbiology and Infection, University of Birmingham, Birmingham, UK, for facilitating the sequencing of the amplimers.

REFERENCES

[1] Woo PCY, Lau SKP, Yuen KY. Macrolides as immunomodulatory agents. Curr Med Chem. Anti-Inflamm. Anti-Allergy Agents. 2002;1:131-41.
[2] Tu D, Blaha G, Moore PB, Steitz TA. Structures of MLSBK antibiotics bound to mutated large ribosomal subunits provide a structural explanation for resistance. Cell. 2005;121(2):257-70.
[3] Leclercq R. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Clin Infect Dis. 2002;34:482-92.
[4] Alli OAT, Ogbolu DO, Shittu AO, Okorie AN, Akinola JO, Daniel JB. Association of virulence genes with mecA gene in *Staphylococcus aureus* isolates from Tertiary Hospitals in Nigeria. Indian J Pathol Microbiol. 2015;58(4):464-71.
[5] Coutinho VS, Paiva RM, Reiter KC, de-Paris F, Barth AL, Machado ABMP. Distribution of erm genes and low prevalence of inducible resistance to clindamycin among staphylococci isolates. Braz J Infect Dis. 2010;14(6):564-68.
[6] Morel CM, Mossialos E. Stoking the antibiotic pipeline. Br Med J. 2010;340:1115-18.
[7] Tacconelli E. Methicillin-resistant *Staphylococcus aureus*: source, control and surveillance organization. Clin Microbiol & Infect. 2009;15(s7):31-38.
[8] Livermore DM. Antibiotic resistance in staphylococci. Int J Antimicrob Agents. 2000;16:S3-S10.
[9] Duran N, Ozer B, Duran GG, Onlen Y, Demir C. Antibiotic resistance genes and susceptibility patterns in staphylococci. Indian J Med Res. 2012;135:389-96.
[10] Ho PL, Johnson DR, Yue AWY, Tsang DNC, Que TL, Beall B, et al. Epidemiologic analysis of invasive and noninvasive group A streptococcal isolates in Hong Kong. J Clin Microbiol. 2003;41:937-42.
[11] Nyasulu P, Murray J, Perovic O, Koornhof H. Antimicrobial resistance surveillance among nosocomial pathogens In South Africa. J Exp Clin Med. 2012;4(1):08-13.
[12] Olowe OA, Kukoyi OO, Taiwo SS, Ojurongbe O, Opaleye OO, Bolaji OS, et al. Phenotypic and molecular characteristics of methicillin-resistant *Staphylococcus aureus* isolates from Ekiti State, Nigeria. Infect Drug Res. 2013;6:87-92.
[13] Adamu JY, Raufu AI, Chimaroke FC, Ameh JA. Antimicrobial susceptibility testing of *Staphylococcus aureus* isolated from apparently healthy humans and animals in Maiduguri, Nigeria. Int J Biomed Health Sci. 2010;6:191-95.
[14] Cheesbrough M. District Laboratory Practice in Tropical Countries Part 2. Cambridge University Press, Cambridge. 2000; pp. 157-66.
[15] Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: Twenty seventh informational supplement M100-S27 CLSI Wayne, PA, USA. 2017; pp. 56-63.
[16] Alli O, Akinloye O, Rowley D, Butcher P. A comparative assessment of ribosomal DNA polymorphisms in methicillin resistant *Staphylococcus aureus* (MRSA) epidemiology. Afr J Biomed Res. 2007;10:117-25.
[17] Sutcliffe J, Grebe T, Tait-Kamradt A, Wondrack L. Detection of erythromycin-resistant determinants by PCR. Antimicrob Agents Chemother. 1996;40:2562-66.
[18] Gerard L, Quaglia A, Reverdy ME, Lequerq R, Vandenesch F, Etienne J. Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among Staphylococci. Antimicrob Agents Chemother. 1999;43(5):1062-66.
[19] ClustalW website. Available from: http://www. ebi.ac.uk/Tools/msa/clustalw2/help/index.html.
[20] Chancey ST, Zähner D, Stephens DS. Acquired inducible antimicrobial resistance in Gram-positive bacteria. Future Microbiol. 2012;7:959-78.
[21] Botrel MA, Haenni M, Morignat E, Sulpice P, Madec JY, Calavas, D. Distribution and antimicrobial resistance of clinical and subclinical mastitis pathogens in dairy cows in Rhone-Alpes, France. Foodborne Pathog Dis. 2010;7:479-87.

[22] Roberts MC, Sutcliffe J, Courvalin P, Jensen LB, Rood J, Seppala H. Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. Antimicrob Agents Chemother. 1999;43:2823-30.
[23] Roberts MC. Update on macrolide-lincosamide-streptogramin, ketolide, and oxazolidinone resistance genes. FEMS Microbiol Lett. 2008;282(2):147-59.
[24] Vitali LA, Petrelli D, Lamikanra A, Prenna M, Akinkunmi EO. Diversity of antibiotic resistance genes and staphylococcal cassette chromosome mec elements in faecal isolates of coagulase-negative staphylococci from Nigeria. BMC Microbiology. 2014;14:106.
[25] Ardic N, Ozyurt M, Sareyyupoglu B, Haznedaroglu T. Investigation of erythromycin and tetracycline resistance genes in methicillin-resistant staphylococci. Int J Antimicrob Agents. 2005;26(3):213-18.
[26] Petrelli D, Zampaloni C, D'Ercole S, Prenna M, Ballarini P, Ripa S, et al. Analysis of different genetic traits and their association with biofilm formation in *Staphylococcus epidermidis* isolates from central venous catheter infections. Eur J Clin Microbiol Infect Dis. 2006;25(12):773-81.
[27] Martineau F, Picard F, Lansac N, Menard C, Roy PH, Ouellette M, et al. Correlation between the resistance genotype determined by multiplex pcr assays and the antibiotic susceptibility patterns of *Staphylococcus aureus* an *Staphylococcus epidermidis*. Antimicrob Agents and Chemother. 2000;44:231-38.
[28] Schmitz FJ, Sadurski R, Kray A, Boos M, Geisel M, Kohrer K, et al. Prevalence of macrolide-resistance genes in *Staphylococcus aureus* and *Enterococcus faecium* isolates from 24 European university hospital. J Antimicrob Chemother. 2000;45:891-94.
[29] Van BF, Balzi E, Tulkens PM. Antibiotic efflux pumps. Biochem Pharmacol. 2000;60:457-70.
[30] Ding ZF, Zhang H, Tang W, Tong CY, Li RT, Chen LX, et al. Methylase Genes-Mediated Erythromycin Resistance. Isr J Vet Med. 2012;67(3):170-79.

PARTICULARS OF CONTRIBUTORS:
1. Senior Lecturer, Department of Biomedical Science, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo, Nigeria.
2. Reader, Department of Biomedical Science, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo, Nigeria.
3. Graduate Assistant, Department of Biomedical Science, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo, Nigeria.
4. Graduate, Department of Biomedical Science, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo, Nigeria.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. David Olusoga Ogbolu,
Senior Lecturer, Department of Biomedical Science, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo, Nigeria.
E-mail: doogbolu@lautech.edu.ng

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Jun 07, 2017**
Date of Peer Review: **Aug 02, 2017**
Date of Acceptance: **Apr 11, 2018**
Date of Publishing: **Oct 01, 2018**