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16S rDNA Sequencing analysis in identification of some multidrug resistant (MDR) bacterial isolates from clinical specimens

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Abstract

Accurate identification of bacterial pathogens from clinical specimens and Multidrug Resistant (MDR) characterization is a key to empirical therapy. Twelve (12) bacteria isolates from blood, urine and faecal samples were selected based on the ability to grow on Luria Bertani (LB) agar medium containing 100µg/ml ampicillin, identified by 16S rDNA PCR and sequencing. Identified isolates tagged; U01, U02, U03, U04, S08, U10 and U11 were from urine specimens, S05, S06, S07 and S12 from stool, while B09 was from blood. The isolates were screened for MDR pattern according to Kirby-Bauer disc diffusion method. Conventional biochemical tests revealed that all the isolates are *Escherichia coli.* The 16S gene sequencing results confirmed that, ten (10) isolates had high similar sequence alignment with identified *E. coli* strains, while two are *Enterobacter cloacae* and *P. aeruginosa*. The antimicrobial susceptibility pattern shows that, most of the isolates (83.3%) were MDR. All the 12 isolates (100%) are resistant to Ampicillin, Cephalothin, Erythromycin, Fusidic acid, Novobiocin and Oxacillin, but sensitive to Colistin sulphate and Imipenem. Eleven isolates (91.7%) are resistant to Chloramphenicol, Cotrimoxazole, Streptomycin, Sulphatriad and Tetracycline. Eight of the 12 isolates (66.7%) are resistant to Ciprofloxacin and Ceftriaxone. Seven (58.3%) are resistant to Cefotaxime, Cefuroxime and Gentamycin. Nine (75%) are sensitive and three isolates (25%) are resistant to Augmentin. The high resistance to these antibacterial agents in this study was due to the indiscriminate use of first-line common antibiotics like ampicillin in the study area, which is now substituted with Augmentin. Routine biochemical identification tests should always be confirmed with genotypic methods such as 16S gene sequencing, to avoid misdiagnosis, as variations do exist among some bacterial strains.

Keywords: Multidrug resistance, sequencing, 16S rDNA, *E. coli*, *Enterobacter cloacae*, *P. aeruginosa*.
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Introduction

Proper identification of bacterial isolates is a major role of clinical diagnostic microbiology. However, conventional routine laboratory techniques are cheaper and faster in identification of most of the commonly encountered bacterial pathogens, some species are not easily identified. These methods also have shortcomings due to emergence of uncommon bacteria or those species with ambiguous metabolic properties (Munkhdelger *et al.*, 2017). For variable, slow-growing bacteria to be identified, adequate time and standard laboratory working conditions are needed. These requirements are hardly met in our laboratories, due to ill-equipment and poor electricity supply. 16S ribosomal DNA sequencing serve as a universal techniques that basically, provides

solutions to problems encountered in conventional methods, yielding authentic results, even for all types of bacteria species. 16S rDNA sequencing is useful in identification of those bacteria that cannot be easily detected phenotypically. Molecular identification of bacterial isolates from clinical specimens is more sensitive and reliable, as some significant pathogens are missed during conventional routine laboratory screening.

Resistance of bacteria to common antimicrobial agents is a great threat to public health globally. Dissemination of multidrug-resistance among bacterial pathogens is commonly found to be plasmid-mediated. MDR bacterial strains have increased with the application of broad-spectrum antibacterial agents. A large number of drug-resistant genes have been reported (Li *et al*., 2012; Zhou *et al*., 2018). Multidrug resistance have negative impact on the treatment of infections caused by bacterial pathogens from both intestinal and extraintestinal sites. The multiple levels of resistance transmission make elucidation of the antimicrobial chemotherapy of Enterobacteriaceae extremely complicated (Abd El Ghany *et al*., 2018). This study was therefore aim at identifying bacterial isolates from clinical specimens using 16S rDNA analysis and determining the multidrug resistant pattern of the isolates.

Materials and Methods

Phenotypic identification and selection of bacterial isolates

A total of twelve (12) isolates were identified based on the conventional phenotypic characteristics; Gram reaction, indole, citrate, urease, triple sugars and using analytical profile index (API 20E) test strips (Biomurieux, France). The isolates were selected for molecular studies by their ability to grow on Luria-Bertani (LB) agar medium containing 100µg/ml ampicillin (Melford Laboratories, UK), as described by Sambrook and Russel (2001).

Molecular identification of bacterial isolates

Colony PCR amplification of 16S rDNA gene

The isolates were screened by 16S colony PCR for bacterial species identification. A total 25 µl was used as the PCR volume, which comprised of 12.5µl master mix/My Taq polymerase, 10.5µl double distilled water, 1µl each of forward and reverse primers. From overnight culture plate, a sterile pipette tip was used to picked and transfer a single bacterial colony into the reaction tube (0.2ml Eppendorf tube). The PCR was performed in G-Storm thermocycler, GS 00001 (ThermoFisher Scientific, UK). The following PCR reaction conditions was used: Initial denaturation at 94°C for 3minutes, then 35 cycles of denaturation (95°C for 45 seconds), annealing (56°C for 30 sec) and extension (72°C for 1 minute), with a final extension at 72°C for 3 mins. A molecular marker 1 kb DNA Hyperladder (Bioline, UK) was used to assess PCR product size. The products were separated by gel electrophoresis on 1% agarose, stained with gel red and the image were captured digitally with UV transillumination using G:Box Chemi-XX6 (Syngene, UK). The characteristics of the universal primer used for the species identification is given below.

Characteristics of primer for amplification of 16S rDNA in the target bacterial species

Gene	Primer	Oligonucleotide sequence (5˙- 3˙)	Size (bp)	Reference
16SrDNA	UNI F	GAGTTTGATCCTGGCTCAG	1500	Barghouthi, (2011)
	UNI R	GGACTACCAGGGTATCTAAT		

16S rDNA purification, quantification and Sequencing

The 16S rDNA colony PCR products were clean-up using QIAquick PCR purification kit/protocol (Qiagen, UK). The clean products was quantified using Cubit 4 Fluorometer (ThermoFisher Scientific, UK). DNA purity was estimated by Lambda XLS UV Spectrophotometer, L7110191 (Perkin-Elmer Inc., USA). UV absorbance at 260, 280, and 230 was measured according to manufacturers' instructions. The absorbance at 260 nm (A_{260}) and at 280 nm (A_{280}) for the DNA was measured to determine its purity. The quantified DNA was finally analyzed by sequencing techniques for the species identification at the Medical Research Institute, University of Dundee, UK.

Screening of isolates for multidrug resistance (MDR)

The isolates were screened for antimicrobial susceptibility pattern, multidrug resistant characteristics on Iso-sensitest agar (ISA) media (Oxoid, UK), using McFarland standard inoculum according to Kirby-Bauer disc diffusion methods as described by (Mahon *et al*., 2007). The following commercial discs were used: Combined discs M13/M14 rings (Mast Diagnostics, UK): Chloramphenicol (5µg), Erythromycin (5µg), Fusidic acid (10µg), Oxacillin (5µg), Novobiocin (5ugµg), Penicillin (1I.U), Streptomycin (10µg), Tetracycline (25µg), and M14 rings: Ampicillin (10 µg), Cephalothin (5µg), Colistin sulphate (25µg), Gentamycin (10µg), Sulphatriad (200µg), and Cotrimoxazole (25 µg). Single discs (Oxoid): Amoxycillin-clavulanate/Augmentin (30µg), then Imipenem (10µg), Cefuroxime (30µg), Ceftriaxone (30µg), Cefotaxime (30µg), Ciprofloxacin (10µg) and. The diameter zone of inhibition were measured in millimetre and results were interpreted according to British society for antimicrobial chemotherapy (BSAC) guidelines, (2013). *Escherichia coli* NCTC 11560 and *E. coli* ATCC 35401 was used as positive control; while *E. coli* K-12 DH5α as negative control.

Results and Discussion

Antimicrobial drug resistance is a great threat for therapeutic failure in human medicine. The presence of Enterobacteria, especially multidrug resistant *Escherichia coli*, has increased during past decades in terms of the worldwide distribution of resistance traits and evolution of different genes**.** In this study, the conventional biochemical profile revealed that all the isolates are *Escherichia coli.* Isolates U01, U02, U03, U04, U10 and U11 were from urine specimens, S05, S06, S07, S08 and S12 from stool, while B09 was from blood. But when the API 20E test was used to identify the isolates, the results interpretation in the test manual show that three isolates were *E. coli, Enterobacter cloacae* and *Kluvera sp.,* and no identification for the remaining nine isolates. These results indicated that conventional biochemical identification tests should always be confirmed with genotypic methods such as 16S gene sequencing.

Use of 16S rDNA PCR and sequencing for bacterial species identification

The use of 16S rDNA sequencing in clinical microbiology laboratories for bacterial identification, the discovery of novel bacterial genera and species, the detection of uncultivable bacteria and the diagnosis of culture-negative infections are reviewed by some studies (Esparcia *et al*., 2008; Sontakke *et al*., 2009; Barghouthi, 2011). In this study (Figure 5), 16S rDNA species identification by PCR and sequencing confirmed that all the isolates were *E. coli* with the exception of isolates U01 and S08 which were identified as *Enterobacter cloacae* and *Pseudomonas aeruginosa* respectively. A PCR product of 1500 bp was detected for all the isolates under gel documentation system. The variation in the biochemical properties of isolates in this study revealed the importance of molecular identification methods for clinical bacterial isolates in order to avoid misdiagnosis. This is due to the fact that our isolates were carefully identified using conventional biochemical tests as *E. coli* which differ in the case of API 20E test, but confirmed with 16S rDNA sequencing.

Concentration and purity level of DNA extract

After a successful DNA extraction from bacterial isolates, it is a common practice to quantify the DNA prior to using it in downstream applications. To ensure optimal performance, reactions that utilize 16S rDNA or rRNA gene sequencing for bacterial species identification typically specify the required quantities of nucleic acid. In this study (Table 1), purity and concentration of amplified 16S rDNA PCR products from the bacterial isolates was quantified using spectroscopy and cubit fluorometry for species identification by sequencing.

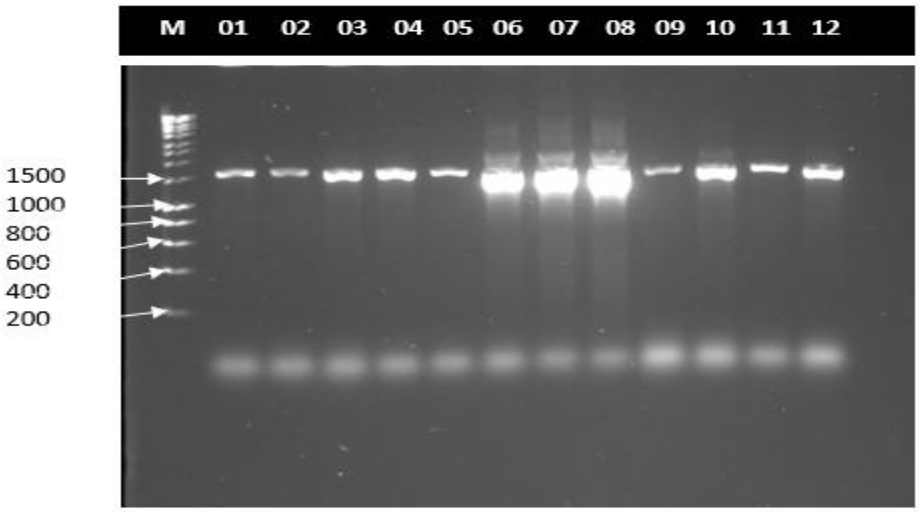

Figure 1: Agarose gel image of 16S rDNA PCR products with amplicon size of 1500 bp specific to Enterobacteriaceae, like *Escherichia coli*. Lane M is a DNA Hyperladder/molecular marker (Bioline, UK).

The absorbance ratio, A_{260}/A_{280} was found to be between 1.4 and 2.8. This shows that the rDNA extracts from eleven isolates (92%) is pure, as it fall within the normal range of high quality DNA (1.8-2.0) (Glasel, 1995). Isolate B09 had low purity level, but with high DNA concentration which is significant for identification. In a study by Ibrahim *et al*. (2014) the ratio of A_{260}/A_{280} was found to be between 1.65 and 1.84, after bacterial DNA purity estimation using NanoDrop technique.

Bacterial strains with 16S gene sequence producing significant alignment

Medical diagnostic microbiology laboratories consider proper identification of pathogenic bacteria as their major tasks. This allow the scientist to differentiate between virulent and avirulent strains, as well as contaminants from mixed cultures, which paves way effective chemotherapeutic intervention, based on patient's underlying clinical condition. In this study (Table 2), the 16S gene sequencing for bacterial species identification revealed that, 10 isolates had high similar gene sequence alignment with the identified *E. coli* strains, while two are *Enterobacter cloacae* and *P. aeruginosa* strains. Eight isolates had 16S rRNA gene partial sequence and four are chromosomal DNA complete genome. Isolate U01 was an *Enterobacter cloacae* strain HFZ-H4 16S ribosomal RNA gene, partial sequence (MG255304.1). *Enterobacter cloacae* is one of the commensal enterobacteria that is highly adaptive to different ecological niche. It is responsible for cases of disease outbreak in a hospital settings as an opportunistic pathogen (Keller et. al., 1998).

E. cloacae has an intrinsic resistance to ampicillin and narrow-spectrum cephalosporins and exhibits a high frequency of mutation to resistance to expanded-spectrum and broad-spectrum cephalosporins. Isolate U03 is *Escherichia fergusonii* strain BI14 16S ribosomal RNA gene, partial sequence (KC986858.1) *E. fergusonii* is a new species in the genus *Escherichia* and family *Enterobacteriaceae* with a 64% homology to *Escherichia coli* after analysis by DNA hybridization techniques. The strain was isolated from cheese, some patients' gall bladder, poultry and livestock faeces. (Glover et. al., 2017).

In this study, 16S gene from isolate U08 has somewhat similar sequence alignment, 89% homology with *P. aeruginosa* R7-539 16S rRNA partial sequence (JQ659890.1). *Pseudomonas aeruginosa* is a MDR bacteria recognized for its ubiquity, its intrinsically high antimicrobial resistance mechanisms, and its association with serious illnesses, nosocomial infections like pneumonia and sepsis. The organism was identified as an opportunistic pathogens, occurring during such disease conditions as cystic fibrosis, traumatic burns and folliculitis.

These infections can be difficult treat due to inherent resistance to antibiotics and adverse contraindications especially with administration of new antimicrobial agents (Klockgether et. al., 2011).

Table 1: Concentration and purity level of the amplified 16S rDNA

Samples	UV Absorbance/O.D (nm)		Absorbance ratio (purity level)	DNA Conc. (ng/µl)
	260	280		
U01	0.032	0.020	1.6	1665
U02	0.017	0.006	2.8	1224
U03	0.023	0.012	1.9	1134
U04	0.088	0.017	2.1	1190
U06	0.024	0.013	1.8	1088
U10	0.054	0.023	1.7	1176
U11	0.032	0.010	2.3	2080
S05	0.090	0.015	2.2	1380
S07	0.043	0.012	2.0	1220
S08	0.025	0.009	2.8	3610
S12	0.058	0.018	2.0	5750
B09	0.088	0.065	1.4	1020

This study found that the isolate B09 from specimen was *E. coli* WG5 chromosomal DNA, complete genome sequence (CP024090.1). *Escherichia coli* strain WG5 is a commonly used bacterial strain used in the identification of phages and as indicator of water pollution by faecal matter. The mutant *E. coli* WG5 is resistant to Nalidixic acid with increased susceptibility to phage infections (Imamovic et. al., 2018).

Isolate U11 was similar to *E. coli* O157:H7 EDL933 with chromosomal DNA, complete genome sequence (AE005174.2). It is a serotype of Shigatoxin-producing *E. coli* (STEC), responsible for foodborne diseases, associated with contaminated dairy products, raw or semi-cooked food. The disease leads to such complications as haemorrhagic colitis, uremic syndrome, and death in young children. (Gally & Stevens, 2017).

Table 2: 16S DNA sequence producing significant alignment for species Identification

Isolate	Identified strain	Gene	Type of genome	Sequence ID	% ID
U01	*Ent. cloacae* HFZ-H4	16S rRNA	partial sequence	MG255304.1	97
U02	*E. coli* isolate 32c	16S rRNA	partial sequence	LK985352.1	97
U03	*E. fergusonii* BI14	16S rRNA	partial sequence	KC986858.1	85
U04	*E. coli* STEC299	cDNA	complete genome	CP022279.1	99
S05	*E. coli* RCB250	16S rRNA	partial sequence	KT260462.1	97
S06	*E. coli* AR-0081	cDNA	complete genome	CP027534.1	98
S07	*E. coli* PD3	16S rRNA	partial sequence	FR715025.1	98
S08	*P. aeruginosa* R7-539	16S rRNA	partial sequence	JQ659890.1	89
B09	*E. coli* WG5	cDNA	complete genome	CP024090.1	96
U10	*E. coli* BJ11203	16SRNA	partial sequence	KP235234.1	97
U11	*E. coli* O157:H7 EDL933		complete genome	AE005174.2	98
S12	*E. coli* RCB250	16S rRNA	partial sequence	KT260462.1	99

These are the highest % ID given in the NCBI data base after blasting. The sequence ID numbers can be used to confirm.

Multidrug resistant characteristics of the isolates

Multidrug resistance in bacteria is most commonly associated with the presence of plasmids which contain one or more resistance genes, each encoding a single antibiotic resistance phenotype. In the present study, table (3) All the twelve isolates (100%) are resistant to Ampicillin, Cephalothin, Erythromycin, Fusidic acid, Novobiocin and Oxacillin. Eleven isolates (91.7%) are resistant to Chloramphenicol, Cotrimoxazole, Streptomycin, Sulphatriad and Tetracycline. Eight isolates (66.7%) are resistant to Ciprofloxacin and Ceftriaxone. Seven isolates (58.3%) are resistant to Cefotaxime, Cefuroxime and Gentamycin. Nine (9) isolates (75%) are sensitive and three (3) isolates (25%) are resistant to Augmentin. However, the isolates were sensitive to Colistin sulphate (100%), Imipenem (100%), and Augmentin (75.0%). The *E.coli* isolates U03, U04, S05, S06, S07, B09, U10, U11 and S12 were sensitive to Augmentin and at least five of the isolates are also sensitive to Cefotaxime, Ceftriaxone, Cefuroxime and Gentamycin. The result showed higher resistance of the isolates to beta-lactams like ampicillin which alarms us that such drugs should no longer be used as first line of treatments in this area, as it used to be in the previous decades. These results of ampicillin-resistant *E.coli* are congruent to the report of Farshad et. al. (2012), who found 100% resistance of their *E.coli* isolates to ampicillin.

A maximum resistance among *E. coli* species isolates was observed against cefpodoxime 100% by Sahu et. al. (2011).The presence of multidrug resistance in this study may be related to the dissemination of antibiotic resistance among hospital isolates. Lim et. al. (2009) observed that *Escherichia coli* are one of the main bacterial pathogens responsible for nosocomial infections especially in immunocompromised patients. The sensitivity of *E. coli* isolates to imipenem was also very high as reported in a previous study by Farshad et. al. (2012). This signifies that this drug can be used as the best option against Uropathogenic *E. coli* (UPEC).

Urinary tract infection (UTI) is one of the commonest infectious disease of human population, caused by multidrug resistant *E. coli* (MDREC) (Hadifar et. al., 2017). In this study (Table 3), isolates from various cases of UTI had the highest prevalence of MDR to commonly used antibiotics, including the third and fourth generation cephalosporins. In a study by Munkhdelger et. al. (2017) in Mangolia, the most common antibiotic resistance was to Cephalothin (85.1%), ampicillin (78.4%) and the least to nitrofurantoin (5.4%) and imipenem (2%), where 93.9% of the isolates were multidrug resistant. The antibiotic resistance profiling of UPEC strains in some reports (Lee *et al*., 2016) elucidates that the antimicrobial agents such as chloramphenicol, cefoxitin, Cefepime, ceftazidime might still be used in the treatment UTIs. Jan et. al. (2009) reported a high potentiality of *E.coli* in developing antimicrobial resistance. This study observed an emergence MDR to third-generation cephalosporins. In Nigeria, the emergence of multidrug resistant strains and its variation over the years has been increasing (Aibinu et. al., 2003).

Enterobacter cloacae is clinically important Gram-negative member of the Enterobacteriaceae and emerging pathogen, causing various nosocomial infections. In this study, the Enterobacter isolate (U01), is resistant all antibiotics tested except Colistin sulphate and Imipenem. The similar MDR pattern other isolates from this study should necessitate an investigation to determine whether a limited spread of a particular isolate existed and also to investigate the mode of resistance to new generation of beta-lactam antibiotics. *Enterobacter cloacae* resistant to extended-spectrum cephalosporins was reported as aetiologic agents of disease among neonates in a Greek hospital (Kartali et. al., 2002).

Table 3: Distribution of bacterial isolates according to multidrug resistant pattern

Antibiotics (µg)	**Bacterial isolates and Zone of Inhibition (mm)/Susceptibility pattern**													
	Std. (mm)		**U01**	**U02**	**U03**	**U04**	**S05**	**S06**	**S07**	**S08**	**B09**	**U10**	**U11**	**S12**	**%MDR**
Ampicillin (10)	15		(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(100)
Augmentin (30)	13		(0.0)	(0.0)	(15.3)	(14.2)	(0.0)	(0.0)	(15.7)	(16.1)	(17.8)	(16.2)	(13.0)	(23.5)	(33.3)
Cefotaxime (30)	20	(0.0)	(0.0)	(39.3)	(12.8)	(32.3)	(0.0)	(10.5)	(12.0)	(37.8)	(36.4)	(0.0)	(35.2)	(58.3)	
Ceftriaxone (30)	24	(0.0)	(0.0)	(34.1)	(12.3)	(34.2)	(0.0)	(10.0)	(11.6)	(29.2)	(35.2)	(13.4)	(33.5)	(66.7)	
Cefuroxime (30)	24	(0.0)	(0.0)	(24.2)	(0.0)	(24.4)	(0.0)	(0.0)	(0.0)	(26.8)	(25.5)	(0.0)	(28.0)	(58.3)	
Cephalothin (5)	15		(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(100)
Chloramphenicol (25)	21		(0.0)	(16.4)	(21.3)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(11.8)	(0.0)	(0.0)	(91.7)
Ciprofloxacin (5)	17		(0.0)	(0.0)	(0.0)	(0.0)	(30.8)	(0.0)	(0.0)	(0.0)	(32.2)	(36.5)	(0.0)	(26.1)	(66.7)
Cotrimoxazole (25)	16		(8.1)	(0.0)	(14.2)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(100)
Colistin sulphate (25)	14		(16.5)	(17.8)	(20.3)	(16.7)	(16.2)	(17.3)	(18.8)	(17.2)	(19.1)	(17.0)	(17.2)	(19.1)	(0.00)
Erythromycin (5)	17		(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(100)
Fusidic acid (10)	30		(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(100)
Gentamycin (10)	17		(8.7)	(25.6)	(0.0)	(0.0)	(20.2)	(0.0)	(0.0)	(0.0)	(24.4)	(23.6)	(23.1)	(7.4)	(58.3)
Imipenem (10)	17		(29.3)	(33.4)	(36.1)	(32.3)	(30.2)	(27.4)	(33.2)	(31.5)	(33.1)	(35.3)	(32.1)	(30.2)	(0.00)
Novobiocin (5)	16		(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(100)
Oxacillin (5)	15		(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(100)
Penicillin G (1I.U)	15		(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(100)
Streptomycin (10)	15		(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(18.2)	(10.8)	(91.7)
Sulphatriad (200)	14		(0.0)	(0.0)	(29.8)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(91.7)
Tetracycline (25)	30		(0.0)	(0.0)	(14.9)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(100)

Standard zone of inhibition interpreted according to BSAC guidelines, 2013

Pseudomonas aeruginosa is one of the major nosocomial and multidrug resistant (MDR) bacterial pathogen. The diseases are difficult to treat because of the limited susceptibility to antibiotics and the high frequency of an emergence of antimicrobial resistance during treatment leading to adverse contraindications (Aloush et. al., 2006). In this study, isolates S08 is also sensitive to Augmentin, Colistin sulphate and Imipenem, as in the case of *E. coli* and *Enterobacter cloacae* strains from this study; but are resistant to the other 17 antibiotics tested. These findings revealed the importance of beta-lactam inhibitors and carbapenems as a therapeutic options against these bacteria.

Conclusion

This study observed that poor chemotherapeutic approach and self-medication have contributed to the emergence of multidrug resistance in the area. High resistance against the commonly used antibacterials is alarming. Conventional biochemical identification tests should always be confirmed with molecular techniques such as 16S gene sequencing, to ensure accurate diagnosis of bacterial infections.

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