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Genotypic Detection of Disinfectant Resistant Genes among Bacterial Isolate of Surgical Site Infections

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ABSTRACT

Background:disinfectants and effective biosecurity measures will be critically important to control microbial diseases.Disinfectant resistance has the potential to change our way of life from compromising food security The current study aim to investigate the bacterial and resistance profile among surgical site infected patients **Methodology:** Eighty SSI swap from patient with age between (15-70) year, also 80 swap from hospitals environmental were collected in this study during a period of 2 months (September to October,2020). The specimens were collected aseptically. The area around the surgical wound was cleaned with 70% ethyl alcohol and the exudates was collected from the depth of the wound using two sterile cotton swabs. The specimens were processed as soon as it reached the laboratory following the standard laboratory procedures. One of the two surgical specimens, was used for gram staining and other for isolation of bacteria by culture. All specimens were cultured inoculated on Chromogenic agar UTI .The total genomic DNA were extracted using G-spinTM Genomic DNA Extraction Kit [for Bacteria] according to the manufacturer instruction (IntronBio/Korea). PCR was performed to amplify genes. **Results:** The results of culturing showed that Out of 80 clinically specimens 77 (96.25%) were culture positive and 3 (3.75%) were negative culture. The numbers of isolates 53 isolates .The most commonly pathogens isolated with pure cultures were *Staphylococcus aureus* 21 isolates (27.27 %) and *Escherichia coli* 14 isolates (18.18%), followed by *Klebsiella pneumonia* 8 isolates (10.38%) *Pseudomonas aeruginosa* 5 isolates (6.49%), *Enterococcus fecalis* 5 isolate (6.49%). While the mix culture 24(31.16).The isolation results of 80 hospitals environmental specimens was 43 (53.75%) were culture positive and 37 (46.25%) were culture negative. The numbers of isolates 39 isolates. The most commonly isolated pathogens with pure cultures were *Staphylococcus aureus* 10 isolates (23.25 %) and *Escherichia coli* 8 isolates (18.60%), followed by *Pseudomonas aeruginosa* 14 isolates (32.55%),*Enterococcus fecalis* 5 isolate (11.62) , *Klebsiella pneumoniae* 2 isolates (4.65%) , Mix cultures 4 (9.30 %).The genotype result of study bacterial isolate of disinfectant resistant showed that *S.aureus* percentage resistant of gene (*qacA, qacE, SugE, mdfA, ydgF*) (57.1% , 100% , 90.4% ,100% , 100%). *E.fecalis* percentage resistant of gene (*qacA, qacE, SugE, mdfA, ydgF*) (40% , 100% , 100% ,100% , 100%). *E.coli* percentage resistant of gene (*qacΔE1, qacE, CepA, SugE, mdfA, ydgF*) (100% , 64.2% , 71.4% ,100% ,100% , 100%). *K.penumonia* percentage resistant of gene (*qacΔE1, qacE, CepA ,SugE, mdfA, ydgF*) (75% , 100% , 100% ,100% , 100% , 75%). *P.aurogenosa* percentage resistant of gene (*qacΔE1, qacE,CepA , SugE, mdfA, ydgF*) (100% , 100% , 100% ,100% , 100% , 100%).

Keywords:surgical site infection , E. coli, P. aerogenes, S. aureus, E. faecalis

Introduction

Surgical Site Infections (SSIs) are defined as infections afflicting either the incision or deep tissue at the operation site. These infections occur within one year of a surgical procedure with an implant and within 30 days without any left implant. They are further categorized in terms of anatomic location: superficial infections afflict only skin or subcutaneous tissue around the incision; deep infections afflict deep soft tissue such as fascia and muscles; organ space infections involve any part of the body, excluding the skin incision, fascia or muscle layers, that is opened or manipulated during the operative procedure [1,2]. The commonest organism causing wound infections was *Staphylococcus aureus*

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followed by other Gram-negative bacilli (*E.coli and Klebseilla and Pseudomonas Spp*)[3]. and *Enterococcus fecallis*These infections are difficult to treat because of the pathogens with increasing antibiotic resistance [4].Hospital-acquired infections are an important health problem . 5 - 10 % of the patients admitted to the hospital, a hospital-acquired infection is observed during their treatment.[5] In recent years, hospital acquired infections caused by multidrug-resistant bacteria increase morbidity and mortality in patients. The transmission of this microorganism is mostly by cross-carriage through the hands of people who are in close contact with the patient, such as healthcare professionals and their relatives.[6] .The main purpose of using disinfectantsin hospitals is to reduce the risk of sporadic and epidemicinfections. To achieve this goal, differentmethods and various disinfectants are recommended[7,8].

In clinical practice, the important hygienic prevention of bacterial pathogen spread is disinfection of potentially contaminated area, such as rooms, utensils and hands.Some antiseptics and disinfectants, sharing common characteristics[9]

The sensitivity of microorganisms to disinfectants varies depending on their structural features. While natural resistance is generally related to the reduction of the disinfectant uptake into the cell; The acquired resistance develops through chromosomal mutations and plasmids[10].Increased resistance of microorganisms to antibioticsand disinfectants and emergence of biocide-resistantbacterial strains have made it difficult to eliminate them.Disinfectants are effective in reducing health care associatedinfections, but the correct choice of disinfectantsconsidering the wide range of efficacy against hospitalpathogens is important and essential in this process [11,12]Bacteria can develop resistance to disinfectants over time either by acquisition of exogenous mobile genetic elements or through the process of intrinsic genetic adaption. Intrinsic genetic alterations such as mutations or differential gene expression brought about due via antimicrobial stress, can significantly contribute to disinfectant resistance[13].

Materials and Methods

Sample collection:

Eighty SSI swap from patient with age between (15-70) year, also 80 swap from hospitals environmental were collected in this study during a period of 2 months (September to October,2020). The specimens were collected aseptically. The area around the surgical wound was cleaned with 70% ethyl alcohol and the exudates was collected from the depth of the wound using two sterile cotton swabs.

Culturing and Identification:

The specimens were processed as soon as it reached the laboratory following the standard laboratory procedures. One of the two surgical specimens, was used for gram staining and other for isolation of bacteria by culture. All specimens were cultured inoculated on Chromogenic agar UTI [14,15].

DNA extraction and PCR:

The total genomic DNA were extracted using G-spinTM Genomic DNA Extraction Kit [for Bacteria] according to the manufacturer instruction(IntronBio/Korea). PCR was performed to amplify genes as mentioned in table (1)

Table 1. Primer Pair Sequences and Amplicon Size andPCR condition of primer

Gene	5-3 sequence	Product	Annealing (°C)
E.coliuida	F : TGGTAATTACCGACGAAAA	162	60.9

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	R :ACGCGTGGTTACAGTCTTGCG-		
K.p.tyrB	F:GGCTGTACTACAACGATGAC R: TTGAGCAGGTAATCCACTTTG	931	60.9
Ps.spp	F: GACGGGTGAGTAATGCCTA R: CACTGGTGTTCCTTCCTATA	618	56
Stap 16S	F:CCTATAAGACTGGGATAACTTCGGG R:CTTTGAGTTTCAACCTTGCGGTCG	791	56
ddl fecalis	F: ATCAAGTACAGTTAGTCTT R: ACGATTCAAAGCTAACTG	941	84.7
CepA	F: CAACTCCTTCGCCTATCCCG R: TCAGGTCAGACCAAACGGCG	1051	61.3
QacA	F: GCTGCATTTATGACAATGTTTG R: AATCCCACCTACTAAAGCAG	629	54.8
QacE	F: GCCCTACACAAATTGGGAGA R: TTAGTGGGCACTTGCTTTGG	350	57.2
YdgF	F: TAGGTCTGGCTATTGCTACGG R: GGTTCACCTCCAGTTCAGGT	330	59.5
MdfA	F: GCATTGATTGGGTTCCTAC R: CGCGGTGATCTTGATACA	284	54.3
SugE	F: CTGCTGGAAGTGGTATGGG R: GCATCGGGTTAGCGGACT	226	58.7
qacΔE1	F: AATCCATCCCTGTCGGTGTT R: CGCAGCGACTTCCACGATGGGGAT	175	56
QacE	F: AAGTAATCGCAACATCCG R: CTACTACACCACTAACTATGAG	258	50

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Ethical Approval :
A valid consent was achieved from each patients before their inclusion in the study

Results and Discussion .

Out of 80 clinically specimens 77 (96.25%) were culture positive and 3 (3.75%) were negative culture. The numbers of isolates 53 isolates.The most commonly pathogens isolated with pure cultures were *Staphylococcus aureus* 21 isolates (27.27 %) and *Escherichia coli* 14 isolates (18.18%), followed by *Klebsiella pneumonia* 8 isolates (10.38%) *Pseudomonas aeruginosa* 5 isolates (6.49%), *Enterococcus fecalis* 5 isolate (6.49%). While the mix culture 24(31.16) (Table 2).The isolation results of 80 hospitals environmental specimens was 43 (53.75%) were culture positive and 37 (46.25%) were culture negative. The numbers of isolates 39 isolates. The most commonly isolated pathogens with pure cultures were *Staphylococcus aureus* 10 isolates (23.25 %) and *Escherichia coli* 8 isolates (18.60%), followed by *Pseudomonas aeruginosa* 14 isolates (32.55%),*Enterococcus fecalis* 5 isolate (11.62) , *Klebsiella pneumoniae* 2 isolates (4.65%) , Mix cultures 4 (9.30 %).Table (3). The results of identification by culturing were as following, for *S. aureus* : manitol salt agar (yellow colony), blood agar (colony with β hemolysis), chromogenic agar (white colony), both catalase and coagulase test was positive and G+ve with cocci in grape-like clusters shape ; for *E. coli* : MacConkey agar (pink colony), blood agar (colony with β+/- hemolysis), EMB agar with green metallic sheen and chromogenic agar (pink colony lactose fermented) , for *P. aeruginosa* : MacConkey agar (colorless colonies , not lactose fermented), blood agar (colony with β +/- hemolysis), EMB agar with white to light pink and chromogenic agar (florescent green colonies), for *K. pneumoniae* :MacConkey agar (pink colonies, lactose fermented), blood agar (colony with β - hemolysis),EMB agar with mucoid pink colonies and chromogenic agar (purple colonies).*E.fecalis*appear trquaz colonies on UTI chromogenic agar. Bacterial species were diagnosed using diagnostic genes. figure (1-5).

Table 2. Prevalence of bacterial isolates from surgical site infection positive cultures

Bacterial species		**No. of isolates (%)**	
Pure Cultures	***S. aureus***	21(27.27)	26(33.76) G+ve
	E. fecalis	5(6.49)	
	E. coli	14(18.18)	27(35.05) G -ve
	P.aeruginosa	5(6.49)	
	K. pneumonia	8(10.38)	
Mix cultures		24(31.16)	
Total		77(100)	

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Table 3. Prevalence of bacterial isolates from the hospitals environment positive cultures

Bacterial species		**No. of isolates (%)**	
Pure cultures	***S. aureus***	10 (23.25)	15(34.87) G+ve
	E.fecalis	5(11.62)	
	E. coli	8 (18.60)	24(55.80) G –ve
	P. aeruginosa	14 (32.55)	
	K.pneumoniae	2(4.65)	
Mix cultures		4(9.30)	
Total		43(100)	

Figure 1: (1.5 %) Agarose gel electrophoresis at 72 volt for 80 minutes of PCR to uidAE.coli amplicon (162bp) , 1-10 represented E.coli samples, M (DNA marker size (100bp).

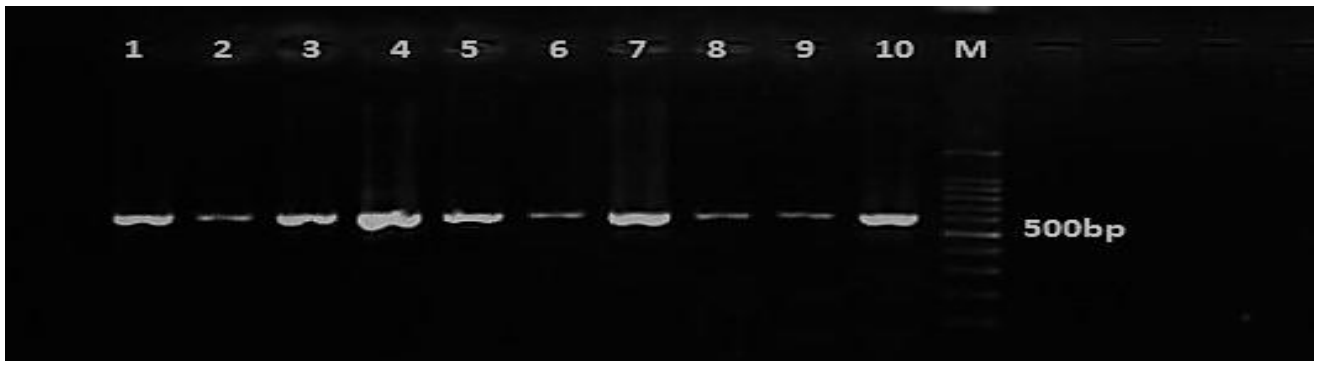

Figure 2: (1.5 %) Agarose gel electrophoresis at 72 volt for 80 minutes of PCR to Ps.spp amplicon (618bp) , 1-10 represented P.aeroginosa samples , MDNA marker size (100bp).

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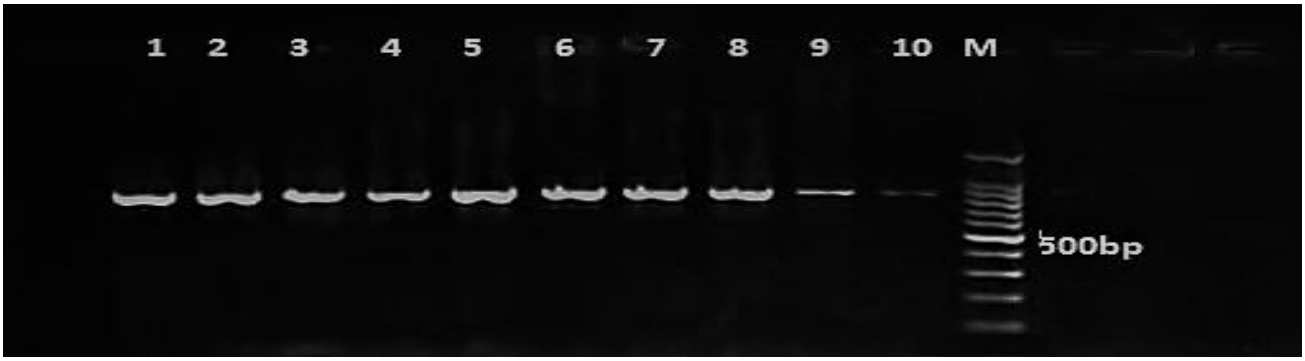

Figure 3: (1.5 %) Agarose gel electrophoresis at 72 volt for 80 minutes of PCR to k.p.tyr B amplicon (931bp) , 1-10 represented K. pneumonea samples , M (DNA marker size (100bp).

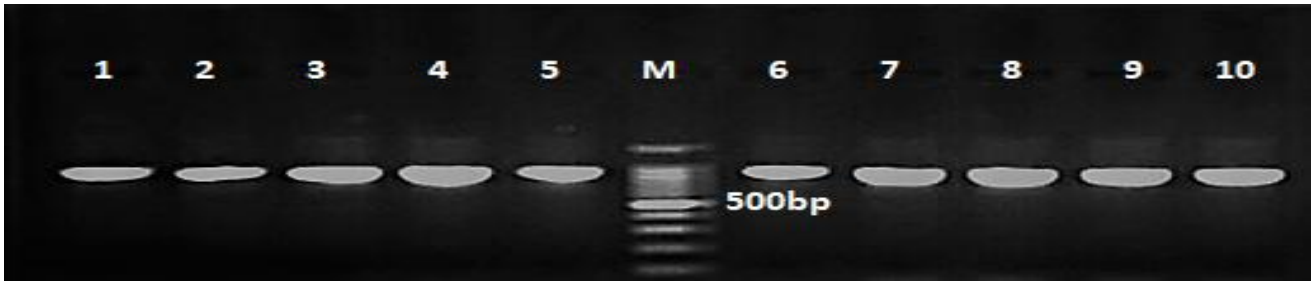

Figure 4:(1.5 %) Agarose gel electrophoresis at 72 volt for 80 minutes of PCR to dd1 fecalis (941bp) , 1-10 represented E. fecalis samples , M (DNA marker size (100bp)

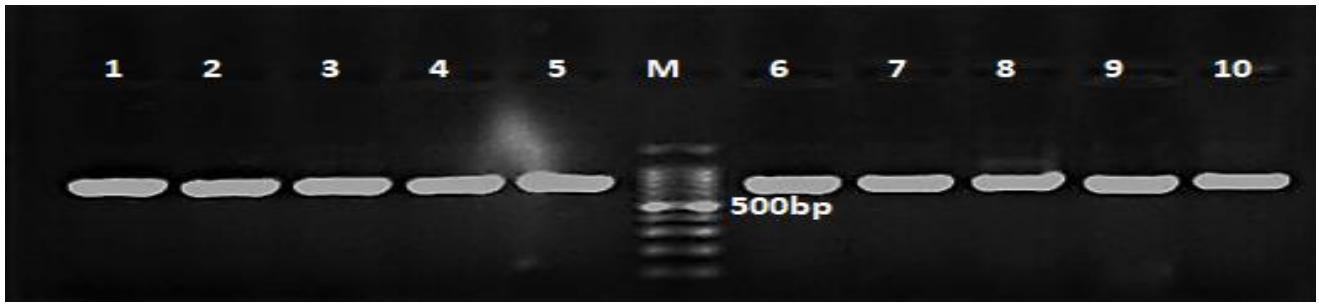

Figure 5: (1.5 %) Agarose gel electrophoresis at 72 volt for 80 minutes of PCR to S.aureus 16S rRNA amplicon (791bp) , 1-10 representedS. aureus samples , M (DNA marker size (100bp).

The genotype result of Clinical bacterial isolate of disinfectant resistant showed tha**t** S.aureus percentage resistant of gene (qacA, qacE, SugE, mdfA, ydgF) (57.1% , 100% , 90.4% ,100% , 100%). E.fecalis percentage resistant of gene (qacA, qacE, SugE, mdfA, ydgF) (40% , 100% , 100% ,100% , 100%). E.coli percentage resistant of gene (qacΔE1, qacE, CepA, SugE, mdfA, ydgF) (100% , 64.2% , 71.4% ,100% ,100% , 100%). This result similar to (Chen,et al., 2020) that show cepA (80%) and disagreement with him qacΔE1 (41%)K.penumonia percentage resistant of gene (qacΔE1, qacE, CepA ,SugE, mdfA, ydgF) (75% , 100% , 100% ,100% , 100% , 75%).this result similar to (Vijayakumar,et al., 2018) that show cepA(100%) and similar to(Goudarzi, et al., 2015) that show qacΔE1 (73%),and disagreement with (Vijayakumar,et al., 2018) that show qacE (11.1%) and that show CepA(56%) and (Chen,et al., 2020) that show qacΔE1 (56%). P.aurogenosa percentage resistant of gene (qacΔE1, qacE,CepA , SugE, mdfA, ydgF) (100% , 100% , 100% ,100% , 100% , 100%).this result similar to (Vijayakumar,et al., 2018) that show CepA (63.3%) and disagreement with him qacE (18.2%) .figure(6-12).

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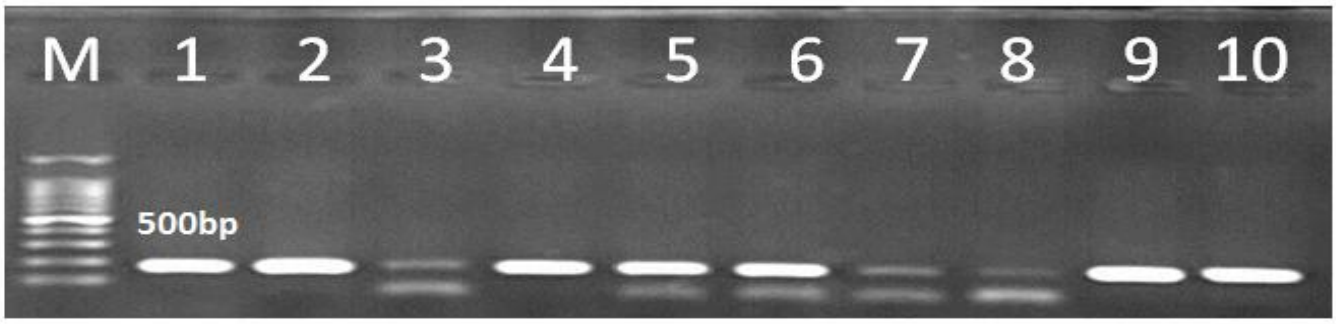


Figure 6:(1.5 %) Agarose gel electrophoresis at 72 volt for 80 minutes of PCR to qacΔE1 amplicon (175bp) , 1-10 represented bacterial isolate , M (DNA marker size (100bp).

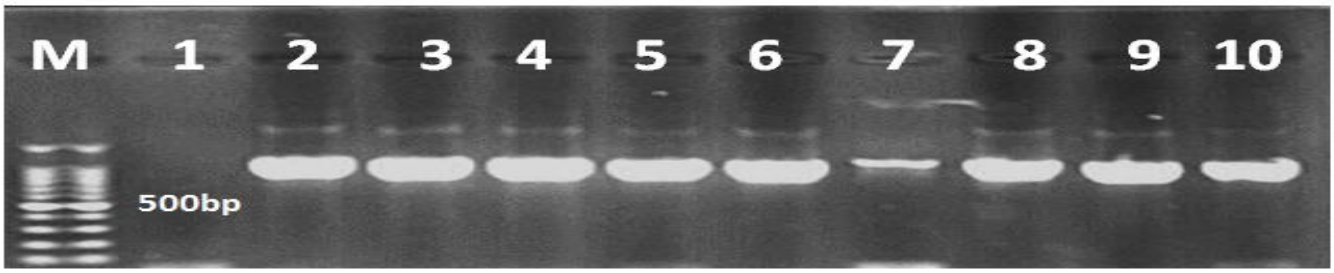
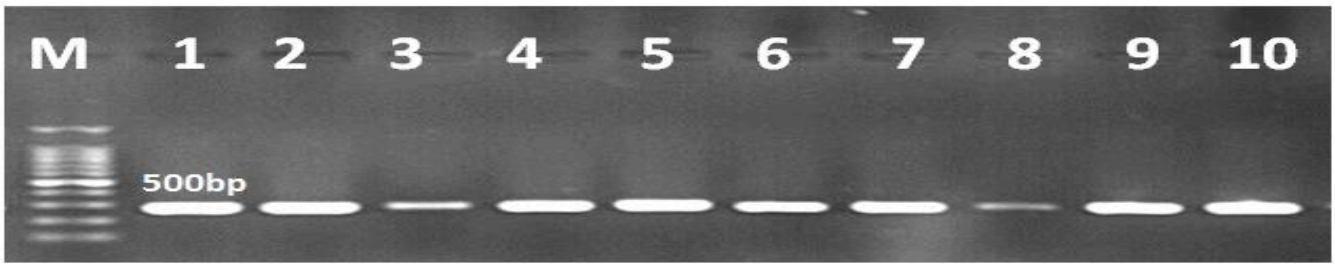
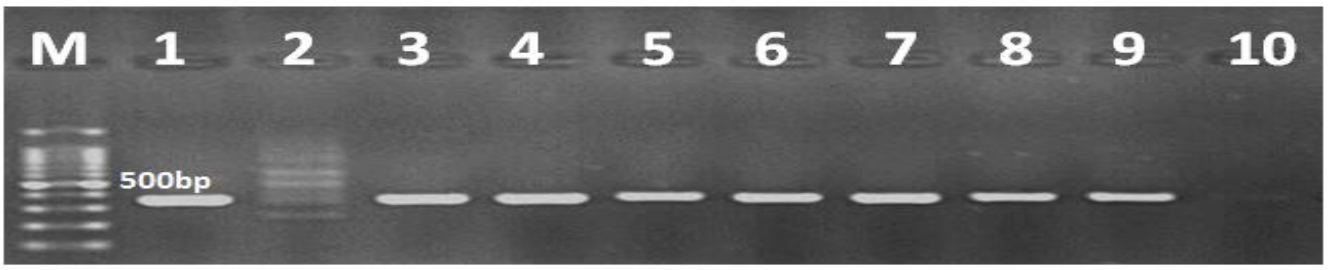


Figure 7:(1.5 %) Agarose gel electrophoresis at 72 volt for 80 minutes of PCR tocepA amplicon



(1051bp) , 1-10 represented bacterial isolate , M (DNA marker size (100bp).

Figure 8: (1.5 %) Agarose gel electrophoresis at 72 volt for 80 minutes of PCR to mdfA amplicon (284bp) , 1-10 represented bacterial isolate , M (DNA marker size (100bp).



F**igurer 9:** (1.5 %) Agarose gel electrophoresis at 72 volt for 80 minutes of PCR to qacE amplicon (350bp) , 1-10 represented bacterial isolate , M (DNA marker size (100bp).

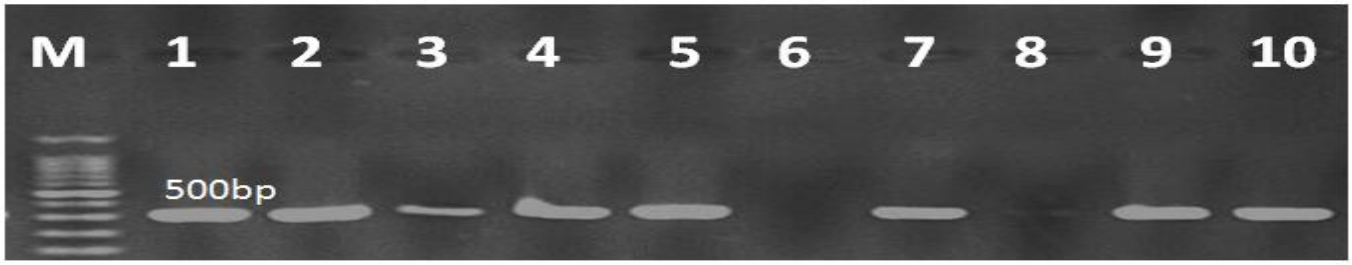


Figure 10:(1.5 %) Agarose gel electrophoresis at 72 volt for 80 minutes of PCR to ydgFamplicon (330bp) , 1-10 represented bacterial isolate , M (DNA marker size (100bp).

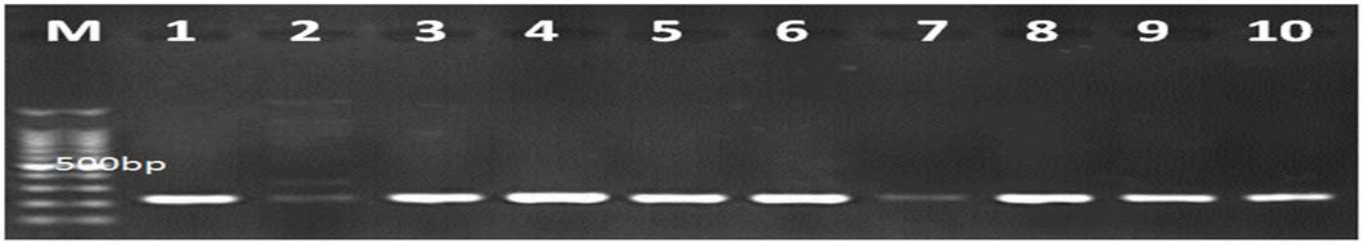
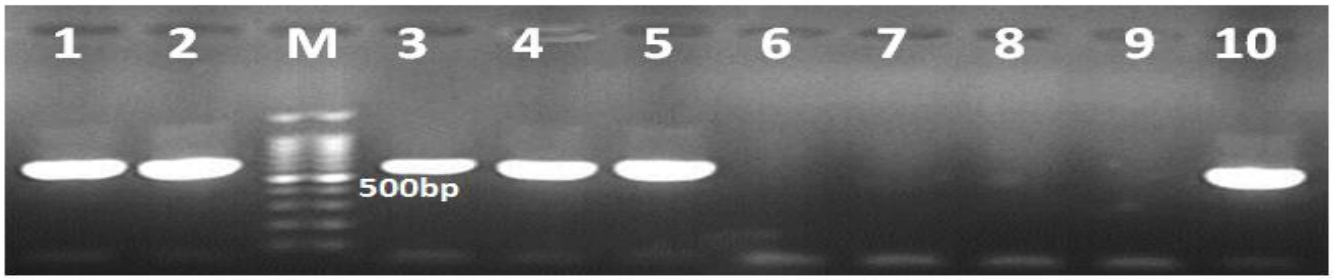


Figure 11:(1.5 %) Agarose gel electrophoresis at 72 volt for 80 minutesof PCR to sugEamplicon



(226bp) , 1-10 represented bacterial isolate , M (DNA marker size (100bp).

Figure 12: (1.5 %) Agarose gel electrophoresis at 72 volt for 80 minutes of PCR to qacA amplicon (629bp) , 1-10 represented bacterial isolate , M (DNA marker size (100bp).

Genes conferring reduced susceptibility to quaternary ammonium compounds are called qac genes. So far, a range of various qac genes have been described and these genes are widely spread among clinical and environmental bacteria [16–18], as seen among two genes (qacA and qacE) which were analyzed in this study. The frequency of qacA and qacE observed in study isolates are coincidental with recent studies performed by Guo et al., where the researchers found 41% qacA and 11.1% qacE in carbapenem resistant K. pneumoniae [19]. This is comparable with recent study conducted by Babaei et al., which while reporting no positive outcomes for qacA, found 73% of tested isolates were carrying qacE genes [18]. However, another study from Iran reported that 40% of A. baumannii clinical isolates were carrying qacE genes [20]. However, many reportshave shownthe frequency of qacAgene to bepredominantly in Gram-positive bacteria and less so in Gram-negative bacteria [16-18]. In relation to the next gene, cepA, this is associated with chlorhexidine resistance in K. pneumoniae and possibly in other related Gram-negative bacteria [21]. This is similar to the findings of Abuzaid et al., conducted in the United Kingdom, where it was reported that 87.5% of K. pneumoniae clinical isolates harbored cepA genes [22]. It is noteworthy that Azadpour et al. reported that the presence of cepA gene in clinical isolates of K. pneumoniae in Iran was 22.4% [23]. Consequently, the distribution and transmission of bacterial reduced susceptibility to biocides is likely to be affected and constrained by biological, physical and socio-economic factors and these vary among different countries, regions and communities. The reason for the difference may be due to variations with the study population, BRGs, of different group of biocides. According to this

observation, a large-scale geographical specific study is required for each specific nosocomial pathogen in order to confirm the reduced susceptibility and presence of BRGs.

Conclusion

Overall, we show here, that a high frequency of BRGs were observed among study isolates .

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