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Original article

Isolation, characterization, and fingerprinting of some multidrug resistance clinical isolates from patients in Al-Qaseem Hospitals, Saudi Arabia

H.S. Alkhowaiter [a,*], Salah E. M. Abo-Aba [a,b]

[a] Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Saudi Arabia
[b] Princess Doctor Najla Bint Saud Al Saud Distinguished Research Centre for Biotechnology, Jeddah, Saudi Arabia

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ABSTRACT

The aim of this study was to investigate the prevalence of antibiotic resistance genes in bacteria isolated from Saudi Arabian patients in the Al-Qassim region. We also examine the application of Random Amplified Polymorphic DNA- Polymerase chain reaction (RAPD-PCR) in genetic diversity of the strains in use. In this study, we have used 29 different clinical samples from Al-Qassim hospitals which consists of wound swabs, blood, sputum and urine. Clinical samples were streaked onto sterile Petri dishes containing nutrient agar media using sterile dry swabs. Additionally antibiotic sensitivity tests, micro scan walkway susceptibility tests and extraction of genomic DNA, amplification of RAPD and Inter simple sequence repeat assay were performed in this study. This study results confirmed from the clinical samples were streaked onto sterile Petri dishes containing nutrient agar media using sterile dry swabs. The PCR analysis in ISSR was confirmed that 30–70% of *E. coli* in Asia and Africa carried ESBLs in the GLASS 2020 study, which is on par with the 59% in VINARES 2016–2017. Comparing VINARES 2016–2017 to other nations, the prevalence of ESBL carriage among *K. pneumoniae* was 35%. However, RAPD results showed that all employed strains and primers could successfully fingerprint DNA. Between isolated bacterial strains, similarity dendrograms were produced. The technique generated bands with the same intensity as the typical PCR carried out using pure DNA, and it worked for all 29 bacterial strains examined. In conclusion, the results show that all of the isolates have at least one antibiotic-resistance gene, and that the PCR approach is a quick, easy, and reliable way to identify genes.

1. Introduction

The misuse of antibiotics has resulted in the quick acceleration and creation of major global problems due to the development of drug-resistant bacteria. Acquired resistance of germs and cancer cells to chemotherapeutic medications with diverse chemical structures and methods of action is known as multidrug resistance (MDR). Overexpression of several proteins responsible for removing the chemotherapic from the cell and reducing its concentration below the effective one leads to multidrug resistance (Catalano, 2022). Antibiotics with higher activity and novel mechanisms of action were produced in greater quantities to fight the resistance challenge. However, various drug-resistant bacteria have emerged and are proliferating at dangerously high levels due to the illogical and incorrect use of antibiotics. The new resistance traits are proliferating at a rate quicker than our\ability to generate new antibiotics, jeopardizing the ability to fight\infectious diseases, surgery and successful organ transplantations. In addition, pharmaceutical companies aren't investing enough time and money into developing new antimicrobial medications, and this is largely due to the low return on investment (Bettiol, 2015; Chawla, 2022).(See Figs. 1-3).

Before humans began mass-producing antibiotics to prevent and treat infectious diseases, many bacterial species had already developed the ability to tolerate them. Resistance mechanisms that predominated before the advent of antibiotics can be deduced from studies of isolated caves, permafrost cores, and other settings and specimens that have been preserved from anthropogenic

* Corresponding author.
E-mail address: hsk996@hotmail.com (H.S. Alkhowaiter).
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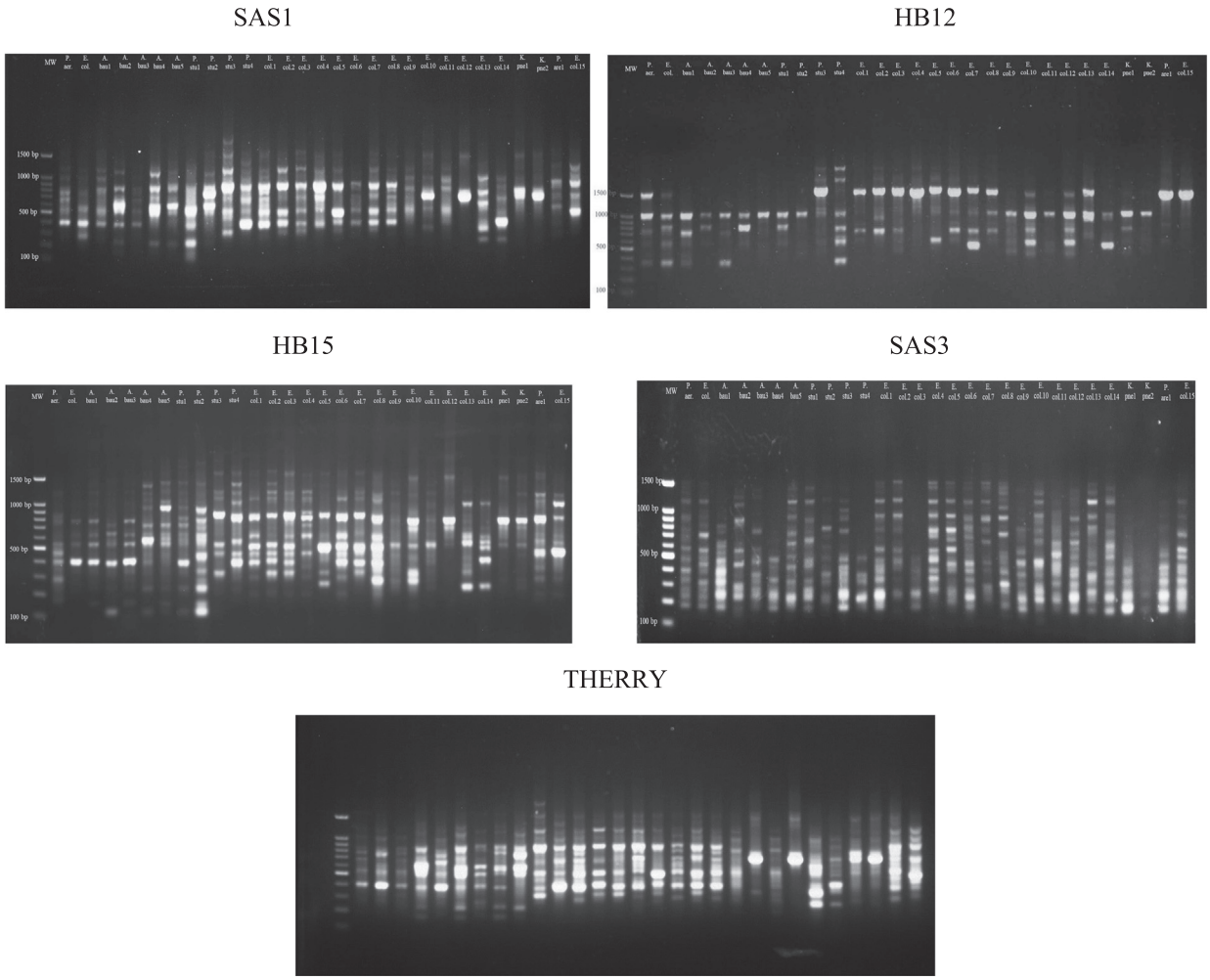

Fig. 1. PCR band profiles generated by SAS1, HB12, HB15, SAS3 and THERRY primers on 1.5% agarose gel electrophoresis with ethidium bromide, M = 1500 bp. P. aer.:C1 ATCC 27853 Pseudomonas aeruginosa, E. coli: C2 ATCC 25922 Escherichia coli, A. bau: Acinetobacter baumannii, P. stu: Providencia stuartii, E. coli1-15: Escherichia coli, K. pne: klebsiella pneumonia, P. aer1: Pseudomonas aeruginosa.

bacterial contamination (Tan, 2018). The constant competition for resources among microorganisms, including the natural production of secondary metabolites that are similar to many of the antibiotics used today in pharmaceuticals, is likely to have been a significant driver of the ancient and still ongoing evolution of resistance mechanisms (Larsson and Flach, 2022). Important in the realm of antimicrobial resistance are antibiotic-resistant bacteria (ARB), the transmission dynamics of which are complicated to decipher due to the presence of resistance determinants and mobile genetic elements in ARB. The prevalence and adaptability of bacteria to survive in different environments under multiple stressors, as well as the fact that antimicrobial resistance genes are carried on broad host range mobile genetic elements that can move between bacterial species, all contribute to this complexity. Traditional epidemiological approaches, which are based on variations in strain characteristics, may incorrectly classify the acquisition of human-associated bacteria from sewage-contaminated environments as transmission between humans, adding another layer of complexity (Leonard, 2022).

In the medical field, the most concerning aspect of these organisms is their high level of aminoglycoside and glycopeptide resistance, as well as their rapid development of resistance to ampicillin, all of which severely limit treatment options (Nowakiewicz, 2017). Members of this family are also linked to the safety of food goods, notably fresh fruit, and constitute part of the idea of microbiological criteria typically used to assess hygiene standards. Epiphytic microorganisms are naturally present on most fresh vegetables, although contamination with potentially human harmful bacteria is still a concern (Richter, 2022).

Mutations play an important role in various microbial productivities, and several researchers have attempted to induce mutation variants with different mutagens in MDR and isolate different mutants with high cellulose production rates (de Paula, 2019). Gene identification is critical for understanding disease pathophysiology and improving diagnosis, prevention, and treatment (Khan, 2015). A mutation is a change in the nucleotide sequence that also applies to single nucleotide polymorphism (Khan, 2021). The Random Amplified Polymorphic DNA (RAPD) and Inter simple sequence repeat (ISSR) techniques can rapidly and accurately discover variation across several different genomes. Using this method, a specific region of the DNA genome is amplified by polymerase chain reaction using primers designed at random (PCR). These banding patterns indicate DNA has been changed (AlSulami, 2019). The current study sought to determine the prevalence of antibiotic resistance genes in bacteria isolated from Saudi Arabian patients in the Al Qassim region. We also examine the application of RAPD-PCR in genetic diversity of the strains in use.

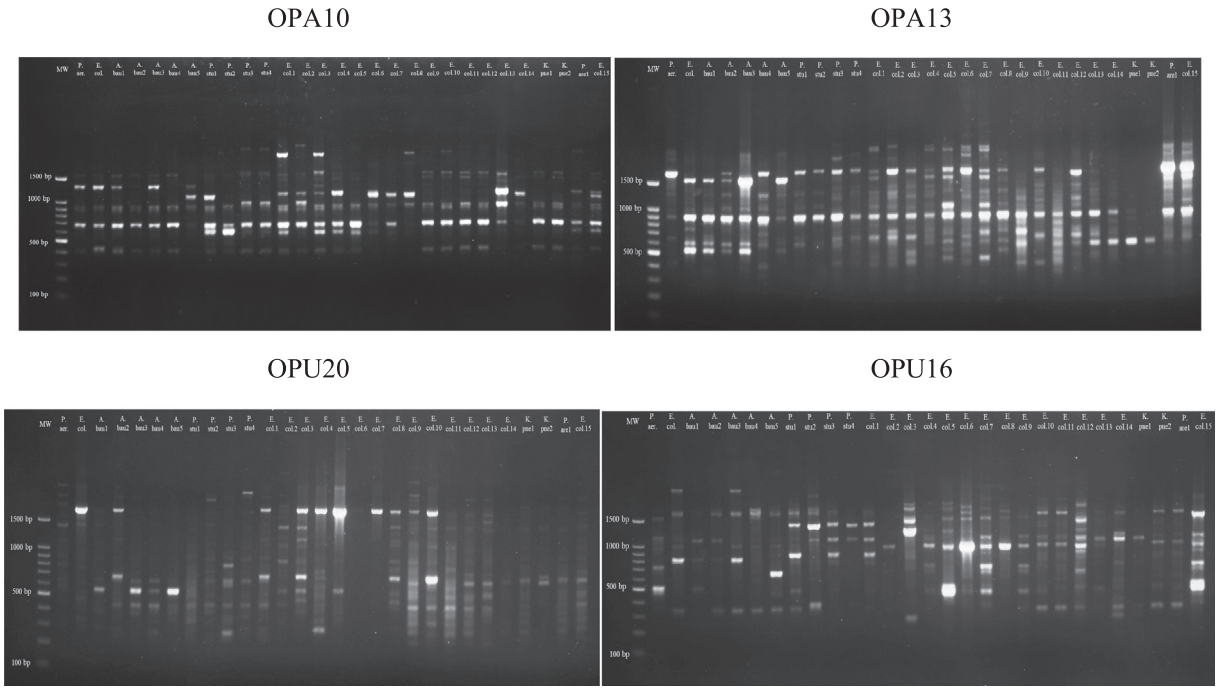

Fig. 2. PCR band profiles generated by OPA10, OPA13, OPU16 and OPU20 primers on 1% agars gel electrophoresis with ethidium bromide, M = 1500 bp. P. aer.:C1 ATCC 27853 Pseudomonas aeruginosa, E. coli: C2 ATCC 25922 Escherichia coli, A. bau: Acinetobacter baumannii, P. stu: Providencia stuartii, E. coli1-15: Escherichia coli, K. pne: klebsiella pneumonia, P. aer1: Pseudomonas aeruginosa.

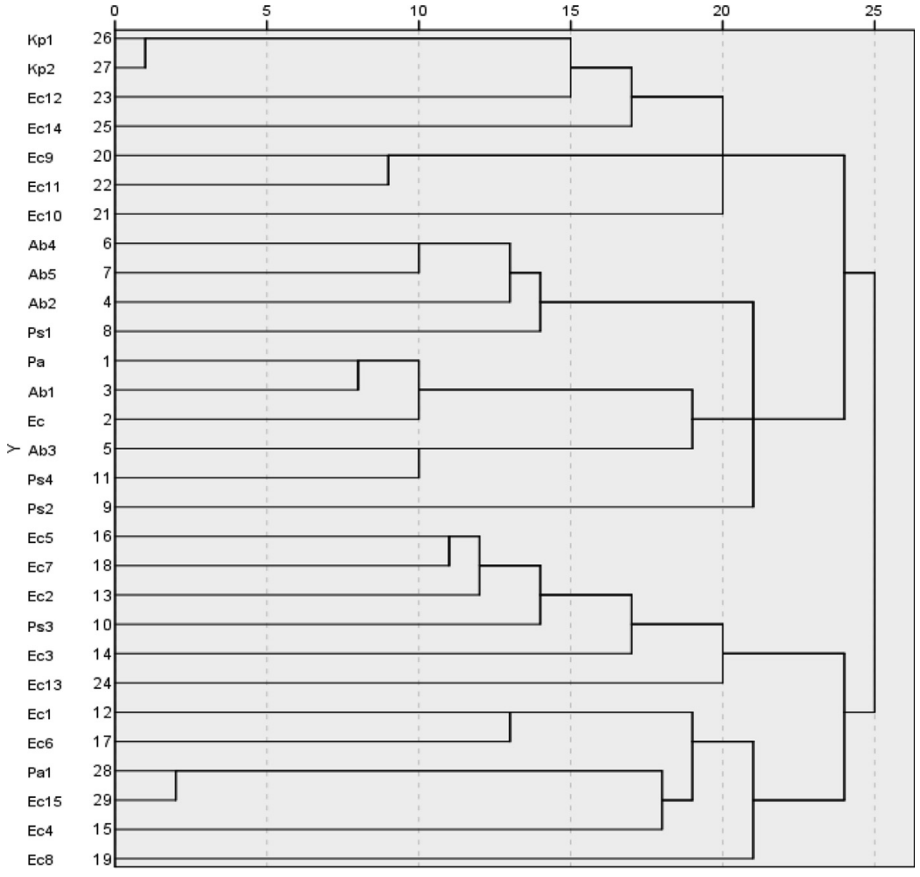

Fig. 3. Dendrogram clustering of variables of 29 pattern bacteria based on all ISSR primers. Cluster analysis of variables is based on their similarity. Pa.:C1 ATCC 27853 Pseudomonas aeruginosa, Ec: C2 ATCC 25922 Escherichia coli, Ab: Acinetobacter baumannii, Ps: Providencia stuartii, Ec1-15: Escherichia coli, Kp: klebsiella pneumonia, Pa1: Pseudomonas aeruginosa.

2. Materials and methods

2.1. Specimens' collection and growth

In this study, 29 clinical samples, including wound swabs, blood, sputum, and urine, were taken from hospitalized patients in the Al-Qassim hospitals in the Kingdom of Saudi Arabia between January and February of 2021. They were chosen from the hospital's inpatient population. Clinical samples were collected in sterile conditions and transmitted right away to the Department of Biology lab, College of Science, King Abdulaziz University, Jeddah, Saudi Arabia.

2.2. Bacterial isolation

Clinical samples were streaked onto sterile Petri dishes containing nutrient agar media using sterile dry swabs (biolife, USA). 48 h were spent incubating the inoculated streaked dishes at 28 °C. Slope into cultures of nutritional agar media after being scooped up by sterile inoculation needles.

2.3. Antibiotic sensitivity test

The isolates' antimicrobial susceptibilities were tested using the CLSI-recommended agar disk diffusion method on Mueller-Hinton agar (Tiantan Biotechnology, PR China). Antibiotic discs (Becton Dickinson, USA) were placed on Mueller-Hinton agar plates and incubated at 37 °C for 24 h. Each zone was measured in millimeters. Penicillin (10 U), erythromycin (15 g), oxacillin (1 g), tetracycline (30 g), gentamicin (10 g), amoxicillin-clavulanic acid (30 g), clindamycin (2 g), vancomycin (30 g), trimethoprim-sulphamethoxazole (25 g), and ciprofloxacin (5 g).

2.4. Determination of minimum inhibitory concentrations (MIC) (Micro scan walkway susceptibility tests)

The manufacturer's recommended MicroScan WalkAway 96 Plus automated ID/AST device evaluated all isolates for antimicrobial susceptibility. The Clinical and Laboratory Standards Institute (CLSI) recommends using the change in antimicrobial concentration to determine the qualitative susceptibility pattern (S: Susceptible, I: Intermediate, R: Resistant) and minimum inhibitory concentration (MIC).

2.5. Genomic DNA extraction

1 ml of each isolated strain's culture was used to extract DNA. To remove the pellet, the culture was centrifuged. The bacterial pellet was gently pipetted with 200 ml of TES buffer (50 mM EDTA, 100 mM Tris 8, and 10% SDS), 20 ml of lysozyme, and 20 ml of proteinase K solution before being incubated at 37 °C for 60 min. 250 μL of sodium acetate 4 M was then added after the pellet suspension had been incubated at 37 °C for 60 min. The mixture was spun down at 10,000 rpm for 5 min. A fresh tube was used to transfer the clear supernatant, and 250 μL of chloroform/isoamyl alcohol was added to the supernatant for 5 min Centrifugation was place for 5 min at 10,000 rpm. The DNA pellet was collected and dried in a laminar flow cabinet for 10 min. Each tube received 50 μL of TE (10 mM Tris pH 8, 1 mM EDTA) (Khan, 2015). By electrophoresis on a 1.5% agarose gel, the DNA was found. Electrophoresis was performed based on the previous study (Khan, 2015).

2.6. RAPD amplification

The fingerprinting of the research sample was detected using a set of five randomly chosen primers, as shown in Table 1. The 25 μL reaction volume utilized for the amplification process included 1 × PCR buffer, 1.5 mM MgCl2, 2 mM dNTPs, 1 U Taq DNA polymerase, 25 μg of template DNA, and 1 μM of each randomly chosen primer. After a first denaturation cycle lasting 5 min at 94 °C, the PCR amplification was carried out in a thermal cycle scheduled to complete 36 cycles. Each cycle was composed of a denaturation phase lasting 1 min at 94 °C, an annealing step lasting 1 min at 32 °C, and an elongation step lasting 1.5 min at 72 °C. In the last cycle, the primer extension phase was prolonged to 10 min at 72 °C. By electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5 μg)) in 1 × TBE buffer at 90 V, the amplification products were separated. Gel documentation system was used to take UV-light-sensitive photos of the gel (Saif and Khan, 2022).

2.7. ISSR assay

The five ISSR primers that were utilized to identify the fingerprints of the research sample are shown in Table 2. The 25 μL reaction volume utilized for the amplification process included 1x PCR buffer, 1.5 mM MgCl2, 2 mM dNTPs, 1 U Taq DNA polymerase, 25 mg of template DNA, and 1 μM of each randomly chosen primer. An initial denaturation cycle lasting 5 min at 94 °C was followed by 36 cycles of PCR amplification in a thermal cycle that was planned to complete them. Each cycle was composed of a denaturation phase lasting 1 min at 94 °C, an annealing step lasting 1 min at 44 °C, and an elongation step lasting 1.5 min at 72 °C. In the last cycle, the primer extension section was prolonged to 10 min. at 72 °C. By electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5 μg) in 1xTBE buffer at 90 V, the amplification products were separated. Gel documentation system was used to take UV-light-sensitive photos of the gel.

2.8. Data analysis

An analysis to determine the genetic relationship between the investigated microorganisms, RAPD data were scored for presence (1) or absence (0) of the bands using Syngene's Gene Tools program. The Jaccard's coefficient was used to calculate a simple matching coefficient in order to generate a similarity matrix. An unweighted average pair group method-based dendrogram and

Table 1
The names and sequences of the primers used in this study for RAPD amplification.

No.	Name of primer	Seq.
1	OPA-10	GTGATCGCAG
2	OPA −13	CAGCACCCAC
3	OPA- 18	AGGTGACCGT
4	OPU-10	ACCTCGGCAC
5	OPU-16	CTGCGCTGGA
6	OPU-20	ACAGCCCCCA

OPA and OPU indicates Variable capacity.

Table 2
The names and sequences of the primers used in this study for ISSR.

No.	Name of primer	Seq.
1	HB10	GAGAGAGAGAGAGGA
2	HB15	GTGGTGGTGGC
3	SAS1	GTGGTGGTGGTGGC
4	SAS3	GAGGAGGAGGAGG
5	TERRY	GTGGTGGTGGGTGRC

cluster analysis were produced using the NTSYS-PC Statistical Package (UPGMA).

3. Results and discussion

3.1. Isolation of antibiotic resistance

On nutrient agar media, 29 clinical samples of blood, sputum and urine were collected from patients in Al-Qassim city, Saudi Arabia and analyzed for the presence of pathogenic bacteria. All samples exhibited obvious bacterial growth and were tested for the presence of multidrug resistant bacteria. The antibiotic resistance genes were isolated from 29 multidrug resistant bacteria isolates at random. *E. coli* 0–15, K. Pne 1–2, P. aer 0 and 1, A. bau 1–5, and P. stu 1–4 were assigned to these isolates.

3.2. Antibiotic usage in bacterial isolates

Table 3 consists of list of percentages of antibiotics used in the bacterial isolated used in this study. Antibiotics list consists of Aminoglycosides, Amikacin, Tobramycin and Gentamycin were applied for *E. coli, K. Pne, P. aer. P. stu and A. bau*. Among *E. coli* species, 20% of Amikacin and 15% of both Tobramycin and gentamycin was documented. Simultaneously, 50% of Amikacin and Gentamycin and 100% of tobramycin was used in *K. Pne*. In *P. aer*, 50% of tobramycin, and 50% of Amikacin and gentamycin were used and finally in *A. bau* 40% of Amikacin, 50% of Tobramycin and 100% of Gentamycin were used. Ciproflaxin and Levoflaxin were used in all 4 bacterial isolates and ampicillin were tested only in *E. coli* (90%) and 100% in *K. Pne*. Other antibiotics such as Amoxicillin, Piperacillin and Ampicillin-Sulbactam was used only in *E. coli* (7–55%) and 100% in *K. Pne*. However, Piperacillin was tested only in *P. aer* (50%) and Ampicillin-Sulbactam was tested in 100% of *A. bau*. The other antibiotics such as Cefepime, Ceftazidime, Imipenem, Meropenem, tigecycline, colistin and aztreonam were used only in E. coli and K. Pne and it was not tested completely *P. aer, A. bau and P.stuartii.*

Clinical microbiology laboratories play a crucial role in confirming susceptibility to selected empirical antimicrobial drugs or identifying resistant strains of bacteria. Some bacterial pathogens remain susceptible to empirical therapy because resistance mechanisms have not been found; for instance, Streptococcus pyogenes remains susceptible to penicillin. Species that may have acquired resistance mechanisms necessitate susceptibility testing of individual isolates (Reller, 2009). According to CLSI standards, antimicrobial susceptibility was determined using the Bioanalyse disc diffusion method and nutrition agar plates (Abdulazeem et al., 2021).

One of the previous studies from Saudi Arabia in Taif region has confirmed the similar study findings carried in different samples (Hassan, 2014). A recent study from Viet Nam population, confirmed a total of 42,553 deduplicated isolates, of which 71% were Gram-negative bacteria and 29% were Gram-positive bacteria. ICUs accounted for 21% of the isolates, while invasive infections accounted for 18% of the samples. The most commonly found species were *E coli* and *S. aeu*, with a total of 21% and 11%, respectively. This was followed by *K. pne* (9.1%) and *A. bau* (9%). Sputum was the most common source for the isolation of bacteria (21%), followed by blood (17%), and urine (12%). For Gram-positive bacteria, 73% of the *S. aur* isolates (3,302/4,515) were MRSA; 34% of the *Enterococcus faecium* isolates (99/290) were resistant to vancomycin; and 58% of the *Streptococcus pneumoniae* (663/1,136) were reduced sensitive to penicillin. 59% of Gram-negative *E. coli* and 40% of Gram-negative *K. pneumoniae* produced ESBL, while 29% and 11% were resistant to carbapenems, respectively. ESBL was formed by 59% of Gram-negative E. coli. The percentage of carbapenem-resistant Acinetobacter spp. was 79%, and the percentage of carbapenem-resistant *Pseudomonas aeruginosa* was 45%. 88% of *Haemophilus influenzae* were resistant to ampicillin, and 7% of *Salmonella species* and 15% of *Shigella* species were resistant to fluoroquinolones (Vu, 2021).

3.3. Inter simple sequence repeat (ISSR- PCR)

This study confirms around 30–70% of *E. coli* in Asia and Africa carried ESBLs in the GLASS 2020 study, which is on par with the 59% seen in VINARES 2016–2017. Comparing VINARES 2016–2017 to other nations, the prevalence of ESBL carriage among *K.*

Table 3
N: number of resistant isolates, n: total number of isolates, e. coli: escherichia coli, k. pneumoniae: klebsiella pneumoniae, p. aeruginosa: pseudomonas aeruginosa and p.stuartii: providencia stuartii n/n (%). na: not available.

Percentage antibiotic resistance pattern of bacterial isolates					
Antibiotics	**E. coli, n/N (%)**	**K. pneumoniae, n/N (%)**	**P. aeruginosa, n/N (%)**	**A. baumannii, n/N (%)**	**P.stuartii n/N (%)**
Aminoglycosides					
Amikacin	3/15(20)	1/2 (50)	1/2(50)	2/5(40)	4/4(100)
Tobramycin	2/15(15)	2/2(100)	1/2(50)	3/5(60)	2/4(50)
Gentamicin	2/15(15)	1/2(50)	1/2(50)	5/5(100)	2/4(50)
Fluoroquinolones Ciprooxacin					
Ciprofloxacin	12/15(80)	2/2(100)	1/2(50)	5/5(100)	1/4(25)
Levofloxacin	14/15(90)	1/2(50)	1/2(50)	5/5(100)	2/4(50)
Penicillins					
Ampicillin	14/15(90)	2/2(100)	Not tested	Not tested	Not tested
β-Lactam					
Amoxicillin-clavulanate	8/15(55)	2/2(100)	Not tested	Not tested	Not tested
Piperacillin-tazobactam	1/15(7)	2/2(100)	1/2(50)	Not tested	1/4(25)
Ampicillin-sulbactam	8/15(55)	2/2(100)	Not tested	5/5(100)	Not tested
Cephalosporins					
Cefepime	9/15(60)	2/2(100)	2/2(100)	5/5(100)	3/4(75)
Ceftazidime	9/15(60)	2/2(100)	2/2(100)	5/5(100)	4/4(100)
Carbapenems					
Imipenem	1/15(7)	2/2(100)	1/2(50)	5/5(100)	3/4(75)
Ertapenem					
Meropenem	1/15(7)	2/2(100)	2/2(100)	5/5(100)	4/4(100)
tigecycline					
tigecycline (TGC)	1/15(7)	1/2(50)	Not tested	Not tested	Not tested
colistin (COL)	2/15(15)	2/2(100)	1/2(50)	Not tested	2/4(50)
Aztreonam (ATM)	9/15(65)	2/2(100)	2/2(100)	Not tested	4/4(100)

pneumoniae was 35%, which was lower than the rates seen in both Cambodia (38%) and Nigeria (77%) (Organization, 2021). The presence of *ampR, blashv-12, penA1, penA2, ampC1, ampC2, aac4A-cr,* and *vanR* on plasmid and genome DNA recovered from utilized bacterial strains was validated in a prior study from Saudi Arabia. The data acquired demonstrates how the widespread and hasty antibiotic usage fuels the emergence of MDR strains (Omar et al., n.d.).

One of the parallel studies was compared with ISSR-PCR analysis from Rassin *et al* which concludes as ISSR9 primers have the potential to generate 5 distinct bands (Rassin et al., 2015; Ibrahim, et al., 2021). Another similar study by Salim et al confirmed the similarity matrix between the isolated fungi revealed that *A. parasiticus* and *A. niger* shared 59% as almost similar. ISSR molecular markers are an extremely useful tool for characterizing the genetic similarity of *Aspergillus* genera (Salim, 2019). In a study by Gokarn *et al* (Gokarn and Pal, 2018) confirmed as Iron-chelation therapy could provide a complementary approach to overcome drug resistance in pathogenic bacteria using exogenous siderophores – exochelin-MS and deferoxamine-B – were evaluated for their inhibitory activity against methicillin-resistant *Staphylococcus aureus* and metallo-β-lactamase producers – *Pseudomonas aeruginosa* and *Acinetobacter baumannii* – by disc diffusion, microbroth dilution, and turbidimetric growth assays. *Acinetobacter baumannii, P aeruginosa, S aureus, Klebsiella pneumoniae*, and *E. coli* were tested as type strains to determine how well iron chelators worked against them in research conducted by Thompson *et al* (Thompson, 2012). When tested in both regular and RPMI tissue culture medium, deferiprone, Apo6619, and VK28 all showed growth inhibition; only deferoxamine showed no impact. Furthermore, a bacteriostatic effect of VK28 was shown in time-kill experiments against *S. aureus*. As a result, the use of these novel iron chelators may offer a novel strategy for combating bacterial infections.

It has been claimed that multiplex PCR is an effective method for identifying resistance genes with high sensitivity, high throughput, and speedy results (Rathore, 2018). Antibiotic resistance genes *KPC, OXA-48*, and *qnrB* were found in *E. coli, OXA-48* and *qnrB* were found in *P. vulgaris, mecA, vanA,* and *qnrB* were found in *E. faecalis, mecA, KPC, OXA-48,* and *qnrA* were found in *S. aureus, KPC, NDM-1, OXA-48,* and the pandemics in recent decades produced by these *Enterobacteriaceae* that regulates these genes have brought into sharp focus the critical need for worldwide surveillance systems of antibiotic resistance (Munita and Arias, 2016). Such monitoring programs might be useful for determining the extent of antibiotic resistance in low-resource settings. Antibiotic resistance is a global problem, but studies that combine monitoring and molecular identification can help discover new resistance mechanisms in returning visitors from endemic regions (Lascols, 2013). The PCR amplification products of *HB12* and *SAS1* genes were studied in E. *coli*; *K. Pne*, *P. aer* and *A. spp* isolates. The details of PCR-primers were showed in Figure-1 documents the bands appearing in *HB12* and *SAS1* genes in 29 bacterial isolates selected in this study. This figure documents the list of bands appeared in HB12 gene. The highest number of bands appeared in HB12 gene is 8 in 3 *E. coli* species, 7 bands were appeared in a couple of *E. coli* and *P. stu* species. However, 1–6 bands were appeared in remaining bacterial isolates used in this study. The frequency range varies between 0.17 and 0.41. Defines the dendogram applied for *HB12* gene with 29 bacterial isolates. Table documents the list of bands appeared in SAS1 gene (Data not shown). The maximum 10 bands were appeared in *P. stu2* strain, 9 bands were present in a couple of *E. coli* strains, 8 bands in appeared in a E. coli strain, 7 bands were appeared in *E. coli, P. stu1* and *P. aer* strains. The frequency of bands varies from 0.1 to 0.8. Figure-3 defines the dendrogram clustering of variables of 29

Table 4
List of primers used, their nucleotide sequences, and the overall number of bands generated.

Primer Name	P. aer.	E. coli	A. bau1	A. bau2	A. bau3	A. bau4	A. bau5	P. stu1	P. stu2	P. stu3	P. stu4	E. coli1	E. coli2	E. coli3	E. coli4	E. coli5	E. coli6	E. coli7	E. coli8	E. coli9	E. coli10	E. coli 11	E. coli12	E. coli13	E. coli14	K. pne1	K. pne2	P. are1	E. coli15	Total
OPA10	6	7	7	6	8	6	5	8	9	9	8	8	10	10	9	7	7	6	7	6	8	7	5	8	5	5	6	9	8	210
OPA13	12	10	8	8	11	12	10	6	4	9	8	11	15	13	13	15	15	15	9	8	11	12	12	10	11	2	2	10	10	290
OPA18	8	8	8	6	4	2	3	6	5	2	9	5	6	6	5	1	5	6	6	10	2	6	6	4	3	8	5	8	3	156
OPU10	5	5	8	5	6	6	4	6	10	8	9	6	9	8	7	8	9	9	4	5	5	5	11	6	5	6	4	1	7	185
OPU16	7	5	4	4	5	5	6	7	7	7	3	8	2	7	7	6	7	9	6	9	11	11	11	7	9	4	7	9	9	199
OPU20	10	10	12	10	6	7	5	5	11	7	10	10	12	9	8	5	1	6	11	14	9	12	9	11	7	10	8	13	14	262
Total Bands 1302																														

pattern bacteria based on all ISSR primers. Cluster analysis of variables is based on their similarity.

3.4. Random amplified polymorphic DNA (RAPD-PCR)

The genomic DNA that was taken from the isolated bacterial strains was employed as templates for RAPD-PCR amplification. Six primers were utilized in this investigation. These primers have amplified various PCR result bands. The 6 primers produced diverse band profiles with several amplified DNA fragments, and the number of amplified fragments likewise varied with different primers. According to figure-2, separated species' amplified fragment sizes ranged from around 100 bp to about 1500 bp (2). The order of the primers is OPA10, OPA 13, OPA 18, OPU 10, OPU 16, and OPU 20, depending on how many DNA fragments are produced. Depending on how many DNA fragments each utilized primer generated, different strain arrangements were made. Results showed that all employed strains and primers could successfully fingerprint DNA. Between isolated bacterial strains, similarity dendrograms were produced. The technique generated bands with the same intensity as the typical PCR carried out using pure DNA, and it worked for all 29 bacterial strains examined.

As indicated in Table 4, the six used primers amplified 96 bands and generated 1302 total numbers of bands for each of the 29 examined bacterial isolations. Table 4 lists the 96 amplified bands as follows: 81 polymorphic bands (85.7%), nine monomorphic bands (14.3%), and 4 unique bands. The OPA 13 primer generated the most bands (290), whereas the OPA 18 primer produced the fewest (156). The acquired banding pattern presented significant distinction between all analyzed isolates and showed a high proportion of genetic variation (85.7) among studied isolates. For the purpose of calculating the genetic similarity coefficient and displaying the results, the produced DNA fragments from RAPD-PCR experiments using various primers for all 29 examined bacterial isolations were employed. Between *E. coli 3 and E. coli* 9, there was the most genetic similarity (0.976), while between the study samples Kp2 and Pa1, there was the least genetic similarity (0.24).

A phylogenetic tree was created using the data collected from RAPD (Fig. 4). The 29 isolates were arranged into three groups using a dendrogram. After applying the cluster analysis to the isolates, which were all in three clusters, a dendrogram was created using pair-wise comparisons of polymorphic products (Fig. 4). Separate from the other samples under investigation, the *Ps4, Ec14, and Ec9* isolates constituted a unique isolate. There are 9 isolates in the first cluster (*Pa, Ab1,Ab2, Ec, Ab5, Ab4, Ab3,Ps2 and Ps1*). Two further groups were created from this cluster. There were four isolates in the first group (*Pa, Ab1,Ab2, Ec, Ab5, Ab4 and Ab3*). The genetic difference between *Ec and Ab2* isolates in this group was found to be the shortest, whereas Ab1 isolate was still closely connected. A second set of two isolates was present (*Ps1 and Ps2*). The second cluster, which was split into two groups, had 12 isolates *(Ec14, Ec13, Ec12, Ec11, Ps3 Ec10, Ec1, Ec8, Ec15, Pa1, Kp2 and Kp1*). Three isolates were present in the first group (*Ec14, Ec13, Ec12, Ec11, Ps3 Ec10 and Ec1)*. The *Ec10 and Ec1* isolates, which were closely linked to those of Ps3, had the closest genetic distance. A second set of two isolates was present (*Ec8, Ec15, Pa1, Kp2 and Kp1*). The isolates *Kp1 and Kp2*, which were closely linked to Pa1, had the closest genetic distance. Five isolates (*Ec2, Ec7, Ec6, Ec5, Ec5, and Ec4*) made comprised the third cluster, which was split into two groups. There were four isolates in the first group (*Ec4, Ec5, Ec6 and Ec7*). *The Ec4 and Ec5* isolates, which were closely linked to *Ec6*, had the closest genetic distance. However, just one isolate was present in the second group (*Ec2*).

The most discriminating primers, OPU20 and OPA13, were utilized to construct the dendrogram. Results indicated that the genotypic characterization and identification of our isolated strains

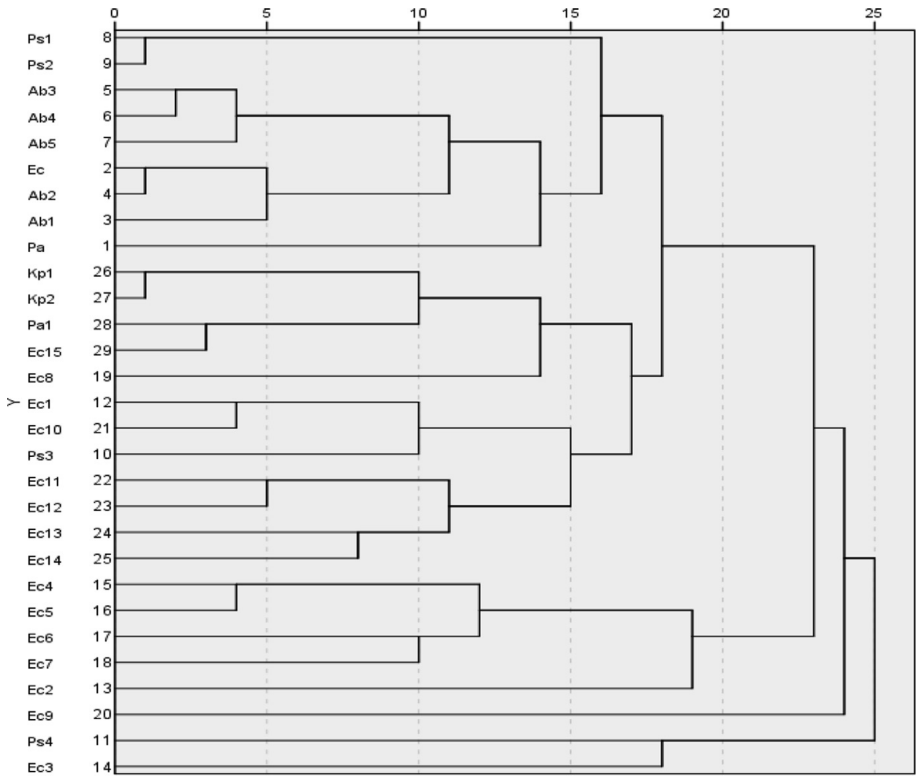

Fig. 4. Dendrogram clustering of variables of 29 pattern bacteria based on all RAPD primers. Cluster analysis of variables is based on their similarity. Pa.:C1 ATCC 27853 Pseudomonas aeruginosa, Ec: C2 ATCC 25922 Escherichia coli, Ab: Acinetobacter baumannii, Ps: Providencia stuartii, Ec1-15: Escherichia coli, Kp: klebsiella pneumonia, Pa1: Pseudomonas aeruginosa.

might be accomplished using genomic fingerprints produced by RAPD. The 29 utilized strains were fingerprinted using PAPD-PCR, and almost all of them revealed a distinctive pattern. The 29 strains utilized in the investigation are also related phylogenetically, as shown. Additionally, RAPD-PCR patterns from the used strains revealed a little variation between them. As a supplement to physiological and biochemical processes, the results revealed that genomic fingerprints derived by RAPD may be employed for the genotypic characterization and identification of strains isolation.

4. Conclusion

The results show that all of the isolates have at least one antibiotic-resistance gene, and that the PCR approach is a quick, easy, and reliable way to identify genes. In addition, 11 primers were used to investigate the relationship between RAPD, ISSR pattern and the differentiation of these 29 isolates. The two molecular markers RAPD and ISSR effectively employed to identify and describe the 29 bacterial strains based on the information gathered from the current investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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