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Polymerase chain reaction amplification of 16S rDNA from three nosocomial bacterial isolates in Kaduna State, Northern Nigeria

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A wide variety of opportunistic pathogens has been detected in hospital surfaces. Medical center surfaces can serve as reservoirs of pathogenic bacteria. Among this pathogens, *Pseudomonas* species are one of the leading causes of nosocomial infections, frequently found in hospital environments. Polymerase chain amplification system remains one of the best methods for the rapid detection of low numbers of pathogenic bacteria with core references to nucleic Acid content. However, there is a limited researches focusing on these techniques to examine the molecular content of nosocomial bacteria. The present investigation examines types and strains of bacteria present in indoor air of wards, fomites and surgical tools of three prominent hospitals namely, Ahmadu Bello University Teaching Hospital Zaria, Hajiya Gambo Sawaba Hospital Zaria City and Barau Dikko Teaching Hospital Kaduna using standard methods. Preliminary Grams reaction and biochemical characterization was done according to standard methods, DNA extraction precede PCR amplification, probable organisms include *Pseudomonas aeruginosa*, *Corynebacterium* sp., *P. aeruginosa*, *Bacillus* sp., *Klebsiella pneumonia*, *Neisseria* sp., *Staphylococcus aureus* and *Staphylococcus epidermidis*. Out of all the isolates that were of public health concern, *Neisseria* sp., *S. aureus* and *P. aeruginosa* are the most prevailing isolates. A strain of *P. aeruginosa* was observed to give a DNA sequence. *P. aeruginosa* was the bacteria isolates sequenced and it showed 100% similarity having the id query: 86603, when blast using National Center for Biotechnology Information (NCBI). In general, patterns were specific at either the genus level or the species level. This research has been able to show that PCR is a promising fast method for the identification of nosocomial microorganisms.

Key words: Polymerase chain reaction, nosocomial bacterial, hospital and DNA.

INTRODUCTION

Nosocomial infection is one of the most common complications within health care facilities, certain studies have reported outbreaks resulting from contaminated

hospital environments, it is called nosocomial infection if it develops 72 h after admission to the hospital (Akihiro et al., 2017). These infections are more dangerous than

other infections because they are caused by bacteria with a high resistance to antibiotics.

Health care facilities provide an environment conducive to exposure and transmission of bacteria; infections caused by these bacteria are an important cause of increased morbidity, mortality and health care costs worldwide (Daniel and Michael, 2015). Nowadays, although modern antibiotics have improved; still sometimes the treatment is difficult and causes morbidity and mortality to patients. Many outbreaks of nosocomial infections have come from reservoirs of pathogens in the inanimate hospital surfaces. The contribution of the environment surfaces remains an important factor in nosocomial infection. It has been reported that majority of the patient acquire this infection while staying in hospital (Ananthan et al., 2011).

The major causative agents in the Western world comprise Gram-positive *Staphylococcus aureus*, *Enterococcus* spp., *Streptococcus* spp., Gram-negative *Escherichia coli*, *Enterobacter* spp., *Proteus mirabilis*, *Klebsiella* spp. In Africa, *P. aeruginosa* are in increasing proportions due to intensive care treatment (Souza et al., 2015). *Pseudomonas* sp. are considered opportunistic pathogens that causes opportunistic infection, they are commonly found in nature (soils, water, plants and animals) and water treatment systems, thus demonstrating their adaptation to environments with low nutrient concentration and over a large temperature range (between 4 and 42°C) (Frickmann et al., 2013). Conventional examination of a bacterial infection mainly relies on culture-based techniques. These cultivations usually yield diagnostic results in days or in some cases up to a week after sampling. Furthermore, cultivation of bacteria is not always successful under laboratory conditions (Kerremans et al., 2008; Yoshimura et al., 2011). Such failures may occur due to unsuitable culturing conditions and methods for the bacterial species under study. Alternatively, the particular patient under investigation may have received antimicrobial therapy before sampling.

Molecular methods based on nucleic acid amplification and hybridization aim to circumvent these problems and hasten diagnostic procedures. In such methods, the pathogen is simultaneously detected and identified, which results in more rapid diagnosis than those obtained by conventional culturing methods and obviates the need for additional culture tests (Akihiro et al., 2017). The purpose of this research was to use molecular techniques as alternative methods for strains level identification of nosocomial bacterial found in indoor air of wards, fomites and surgical tools of three prominent hospitals namely, Ahmadu Bello University Teaching Hospital Zaria, Hajiya Gambo Sawaba Hospital Zaria City and Barau Dikko

Teaching Hospital Kaduna, Northern Nigeria.

MATERIALS AND METHODS

Synopsis of the study location

Kaduna with location Coordinates 10°20'N 7°45'E, and 10.333°N 7.750°E usually referred to as Kaduna state to distinguish it from the city of Kaduna, is a state in Northwest Nigeria. It is the capital of Kaduna with a population of over 6,066,562 (2006 Census). The selected hospitals are the three major government hospitals in the state and the justification for selection of these hospitals was based on high patronization.

Isolation and molecular characterization of bacteria

Bacteria were isolated from indoor air of wards, fomites and surgical tools of Ahmadu Bello University Teaching Hospital Zaria, Hajiya Gambo Sawaba Hospital Zaria City and Barau Dikko Teaching Hospital Kaduna using standard methods described by Kerremans et al. (2008) and Cheesbrough (2006). The bacteria from bed sheets, pillow cases, nurses' desk and surgical tools were isolated using sterile swab sticks while those from the air in surgical wards and main wards were isolated using exposed plate technique.

Media preparation

All the media used in this research work (nutrient agar, nutrient broth, mannitol salt agar and blood agar) were prepared according to manufacturer's instructions.

Sample collection

Sampling sterile swab sticks were immediately introduced into nutrient broth and taken to Kaduna State University Microbiology Laboratory for incubation and further bacteriological analysis. In the exposed plate technique, the agar plates were opened and exposed to the indoor air of the wards for about 5 min. After 5 min of exposure the plates were covered again and taken to Microbiology laboratory for incubation. Both the agar plates and the broth were incubated at 37°C for 24 h. After incubation, the bacterial colonies that showed positive growth were sub-cultured, gram stained and viewed under the microscope using oil immersion x100 objectives lens (Kerremans et al., 2008).

Biochemical characterization and identification of the nosocomial isolate

Bacteria isolates extracted were characterized and identified after studying their Gram reaction as well as cell micro morphology. Other tests performed were spore formation, motility and catalase production, citrate utilization, fermentative utilization of glucose, indole production, methyl red- Voges Proskauer reaction, urease and coagulate production, starch hydrolysis, production of H₂S from triple sugar iron (TSI) agar and sugar fermentation. The test were carried out according to the methods described (Cheesbrough, 2006; Adeoye, 2007; Agwung-Fobellah and Kemajou, 2007; Ochei

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and Kolhatkar, 2008). Microbial identification was performed using the keys provided in Barrow and Feltham (1993) and Bergey's Manual of Determinative Bacteriology (1994).

Molecular characterization of species of bacteria

The bacteria species characterized using biochemical methods were subjected to strain level identification using molecular approach. These involve extracting the DNA using standard protocols, amplifying the DNA using PCR, sequencing the gene of interest using next generation sequencing (NGS).

DNA extraction using phenol chloroform method

DNA was extracted using phenol chloroform standard method as described by Psifidi et al. (2010). 200 µl of the bacterial cells were added in a 1.5 ml Eppendorf tube, 400 µl of lysis buffer (Tris-HCL) and 10 µl of proteinase k was added to the 1.5 ml tube. The tube was vortexed and placed on heat block at 65°C for 1 h while vortexing at interval. 400 µl of phenol chloroform was added and vortexed briefly, it was centrifuged for 10 min at 13,000 rpm to separate the phases. The upper layer was carefully removed with a micro pipette, 400 µl of chloroform was added and vortexed, and was then centrifuged for 5 min at 13,000 rpm. The upper layer was carefully removed, 1000 µl of absolute ethanol and 40 µl of 3 M sodium acetate and mixed by inverting the tube several times.

The tubes containing pure DNA were incubated at -20°C overnight. The tubes were centrifuged for 5 min at 14,000 rpm using cool centrifuge, the upper layer was carefully removed using micro pipette, 400 µl of 70% ethanol was added to the precipitate, the tubes were centrifuged for 5 min at 14,000 rpm using cold centrifuge, the upper layer was carefully removed using micro pipette in order to remove all traces of ethanol, the DNA was then allowed to dry by leaving the tubes open for 20 min at room temperature and 50 µl of sterile water was added, vortexed and kept at -20°C.

PCR amplification of 16S rDNA using conventional PCR

The extracted DNA from different species of bacteria was quantified using Nano drop. Thereafter, the quantified genomic DNA was placed in a tube containing master mix and primer and the tube was spined for 30 s and introduced into a thermocycler. The thermocycler was operated based on initial denaturation (95°C for 5 min), 25 cycles of denaturation (94°C for 1 min), annealing (52°C for 1 min), extension (72°C for 1 min) and final extension (72°C for 7 min) according to Psifidi et al. (2010). 16S rDNA forward sequence 5' GGACTACGGGTATCTAAT 3' and reverse sequence 3' AGAGTTTGATCCAGG 5' after the PCR in the thermocycler, the amplified PCR products of expected size 789 bp were confirmed by agarose gel electrophoresis system. The amplified PCR products was run in 1.5% agarose gel electrophoresis stained with ethidium bromide and was viewed under ultra violet (UV) light (Smith et al., 2003; Black and Foarde, 2007).

Sequencing of the gene

The amplified genes of interest were documented, cut and freeze dried. The freeze dried samples of DNA were sent to Macrogen U.S.A laboratory for sequencing. The genomic sequence data was appropriately analyzed using bioinformatics tools, sequence identification was performed using NCBI Basic Local Alignment Search Tool (BLAST) algorithm, similar sequences were downloaded and aligned while phylogenetic tree was drawn with MEGA 7

software (Kumar et al., 2016).

RESULTS

A total number of 66 bacteria were isolated from three hospitals in Kaduna State namely; Ahmadu Bello University Teaching Hospital, Zaria, Hajiya Gambo Sawaba Hospital, Zaria City and Barau Dikko Teaching Hospital, Kaduna (Table 1). Twenty-two (22) samples were collected from each of the hospitals for preliminary antibiotic resistance potency. Observation shows that only 20 of the bacterial isolates were recorded as multi drugs resistant which includes six (6) from Ahmadu Bello University Teaching Hospital, Zaria, eight (8) from Hajiya Gambo Sawaba Hospital, Zaria City and six (6) from Barau Dikko Teaching Hospital, Kaduna. The morphological and biochemical characterization of nosocomial bacterial isolates from hospital environments was presented in Table 1. Investigation revealed that *S. aureus* occurred as the highest predominant bacterial isolate with the percentage occurrence of 10 (50%) followed by *P. aeruginosa* with 4 (20%) and *Bacillus* sp. had 2 (10%), *Corynebacterium* sp., *Klebsiella pneumoniae*, *Neisseria* sp. and *Staphylococcus epidermidis* all recorded 1 (5%) appearances (Table 1).

DISCUSSION

A number of morphological and biochemical parameters have been used to facilitate the determining of the identities of nosocomial bacteria and other hospital acquired infections (Ateba and Mbeve, 2011). Even though selective and differential media has been used to aid the identification of the bacterial species, yet the sensitivity of these protocols might not be very reproducible between laboratories. Generally, selective and differential media rely on some structural or metabolic property of the species that is preferentially selected. It is highly recommended that they should be combined with confirmatory biochemical and morphological tests.

Table 1 show the morphological and biochemical characteristics of the 20 isolated multidrug resistant nosocomial bacterial (MDRNB). The present study (morphological observation) revealed the cell shapes of 40.00% of the overall isolated microorganisms possessed rod shape under the microscope while 60.00% were found to be cocci in shapes. This is a good characteristic of some organisms but not enough to categorize, hence, all isolates were subjected to grams staining test. About 25.00% of the isolated microorganism from analyzed samples stained pink to red, indicating them to be Gram negative organisms, while 75.00% of the overall isolates stained blue to purple by retaining the crystal violet dye indicating them to be Gram positive organisms (Bergey et al., 1994).

Table 1. Morphological and biochemical features of the selected examined Multi-Drugs Resistant (MDRI) isolate.

Isolate code	Cell shape	Gram reaction	Catalase	TSI reaction	SIM reaction 1 M H ₂ S	C-U	MR	VP	GL	MAL	MNT	SU	LAC	OF OR HL	N-R	Probable organisms
HSP-ABU	LR	-	+	NCNCNC	-+-	+	-	-	NC	NC	NC	NC	NC	OX	+	<i>Pseudomonas aeruginosa</i>
HSP-AGS	MLR	+	-	YGY+	+++	-	+	-	YG	YG	YG	YG	YG	F(G)	+(G)	<i>Corynebacterium</i> sp.
HSP-BDT	SR	-	+	NCNCNC	-+-	+	-	-	NC	NC	NC	NC	NC	OX	+	<i>Pseudomonas aeruginosa</i>
HSP-ABU	MLR	-	+	NCNCNC	-+-	+	-	-	NC	NC	NC	NC	NC	OX	+	<i>Pseudomonas aeruginosa</i>
HSP-AGS	LR	+	++	YGY+	+++	+	+	-	YG	YG	YG	YG	YG	F(G)	+(G)	<i>Bacillus</i> sp.
HSP-BDT	LR	-	++	YGNCNC	+-	+	+	-	YG	YG	YG	YG	YG	F	+	<i>Klebsiella pneumoniae</i>
HSP-AGS	CC	-	+	YGNCNC	+-	-	+	-	YG	YG	YG	YG	YG	F(G)	+	<i>Neisseria</i> sp.
HSP-BDT	MLR	-	+	NCNCNC	-+-	+	-	-	NC	NC	NC	NC	NC	OX	+	<i>Pseudomonas aeruginosa</i>
HSP-AGS	LR	+	++	YGY+	+++	+	+	-	YG	YG	YG	YG	YG	F(G)	+(G)	<i>Bacillus</i> sp.
HSP-ABU	CC	+	+++	YGNCNC	+-	+	+	-	YG	Y	Y	Y	Y	NC	+	<i>Staphylococcus aureus</i>
HSP-AGS	CC	+	+++	YGNCNC	+-	+	+	-	YG	Y	Y	Y	Y	NC	+	<i>Staphylococcus aureus</i>
HSP-BDT	CC	+	+++	YGNCNC	+-	+	+	-	YG	Y	Y	Y	Y	NC	+	<i>Staphylococcus aureus</i>
HSP-BDT	CC	+	+++	YGNCNC	+-	+	+	-	YG	Y	Y	Y	Y	NC	+	<i>Staphylococcus aureus</i>
HSP-BDT	CC	+	++	YGNCNC	+-	+	+	-	YG	Y	Y	Y	Y	NC	+	<i>Staphylococcus aureus</i>
HSP-ABU	CC	+	+++	YGNCNC	+-	-	+	-	YG	Y	Y	Y	Y	NC	+	<i>Staphylococcus aureus</i>
HSP-AGS	CC	+	++	YGNCNC	+-	+	+	-	YG	Y	Y	Y	Y	NC	+	<i>Staphylococcus aureus</i>
HSP-ABU	CC	+	+++	YGNCNC	+-	+	+	-	YG	Y	Y	Y	Y	NC	+	<i>Staphylococcus aureus</i>
HSP-ABU	CC	+	+++	YGNCNC	+-	+	+	-	YG	Y	Y	Y	Y	NC	+	<i>Staphylococcus aureus</i>
HSP-AGS	CC	+	++	YGNCNC	+++	-	+	-	YG	Y	Y	Y	Y	NC	+	<i>Staphylococcus aureus</i>
HSP-AGS	CC	+	++	YGNCNC	+-	+	+	-	YG	Y	Y	Y	Y	NC	+	<i>Staphylococcus epidermidis</i>

CC, Cocci shape; SR, short rod; MLR, medium long rod; LR, long rod; YG, acid and gas production; Y, acid production only; TSI, triple sugar ions; SIM, sulphide, indole, motility tests; CU, citrate utilization test; MR/VP, methyl red and Voges, Proskaur test; GL, glucose; NC, No change; F, fermentative; OX, oxidative; I, indole production; M, motility; H₂S, hydrogen sulphide production; MAL, maltose; MNT, mannitol; SU, sucrose; LAC, lactose; nitrate reduction test; OF, oxidation, fermentation Test; HSP, hospital; ABU, Ahmadu Bello University; HGS, Hajiya Gambo Sawaba; BDT, Barau Dikko Teaching Hospital Kaduna.

PCR amplification of 16S rDNA from the three highest prevailing nosocomial bacterial isolates in Kaduna State

Organisms of concern in this study are *P. aeruginosa*, *S. aureus* and *Neisseria* sp. Figure 1 shows the amplified 16S rDNA gene with band size of 789 bp bands from *Neisseria* sp., *P. aeruginosa* and *S. aureus* obtained from some selected hospitals in Kaduna State. To test whether we can discriminate between these

isolates, *Neisseria* sp., *P. aeruginosa* and *S. aureus* strains were examined for their electrophoretic mobility patterns in PCR amplification (Figure 1). Although the observed differences were small, PCR amplification was capable of distinguishing all the isolates from one and other (Lee et al., 2009). For the *Pseudomonas* species, the observed electrophoretic mobility patterns showed light differences between *Staphylococcus* and *Neisseria* sp. (Figure 1). Because of the closed mobility relativity of all these on

electrophoresis gel, these data suggest species-specific patterns for identification. To evaluate the applicability of PCR amplification as a general tool for the identification of bacteria, the amplified 16S rDNA products from the three nosocomial bacterial isolates were sequenced according to Cole et al. (2009) and the result obtained is shown in Figure 2. Out of the three bacterial isolates analyzed, only one which is *P. aeruginosa* gave a DNA sequence. *P. aeruginosa* was the bacterial isolates sequenced and it showed 100% similarity

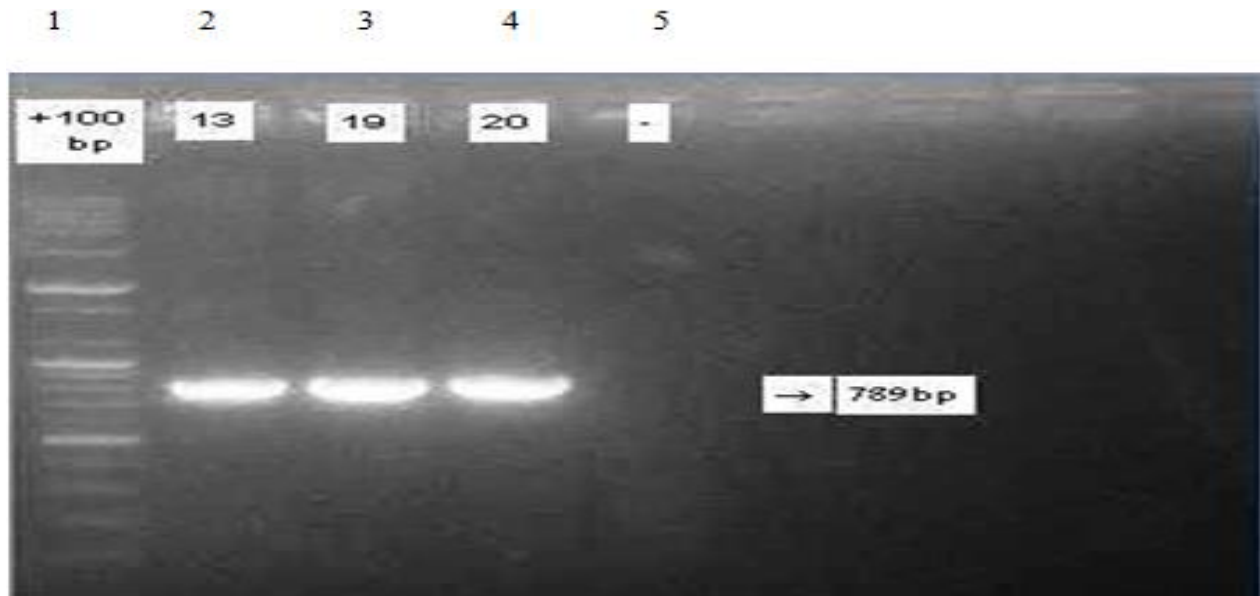


Figure 1. PCR amplification of 16S rDNA gene in three nosocomial bacterial isolates from some selected hospitals in Kaduna State. Lane 1, DNA marker; lane 2, *Neisseria* sp.; lane 3, *P. aeruginos*; lane 4, *S. aureus*; lane 5, negative control.

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51_CTCTCTTGGCGGCGAGTGGCGGACGGGTGAGTAATATATATCGGAACGTGCCAGTAGCGGGGGA
TAACTACTCGAAAGAGTGGCTAATACCGCATACGCCCTACGGGGGAAAGGGGGGGATCGCAAGACC
TCTCACTATTGGAGCGGCCGATATCGGATTAGCTAGTTGGTGGGGTAAAGGCTCACCAAGGCAACG
ATCCGTAGCTGGTTTGGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCAGACTCCTACGG
GAGGCAGCAGTGGGGAATTTTGGACAATGGGGGAACCCCTGATCCAGCCATCCCgcGTGTATGATG
AAGGCCTTCGGGTTGTAAAGTACTTTTGGCAGAGAAGAAAAGGTATCCCCTAATACGGGATACTGCT
GACGGTATCTGCAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGC_31
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Figure 2. Sequence of bacteria isolate (*Pseudomonas aeruginosa*).

having the id query: 86603, when using BLAST National Center for Biotechnology Information (NCBI). The red bullet indicates the sequenced *P. aeruginosa* (Figure 3). In general, patterns were specific at either the genus level or the species level.

However, we opined that in order to overcome lane-to-lane and gel-to-gel differences, PCR resolution was improved by the addition of an internal DNA marker for each sample lane. This makes it feasible to precisely compare relative migration times. This research has been able to show that PCR is a promising fast method for the identification of microorganisms. Also, the use of PCR with universal primers and Single Strand Conformational Polymorphism (SSCP) patterns as an identification method can generally be applied to a wide range of nosocomial bacteria without the need of a large panel of probes.

In order to intensify our research, we added an option for molecular identification of methicillin resistant *S. aureus* species by including the methicillin resistance gene *mecA* in the assay. The identification was based on PCR

amplification as shown in Figure 4. *mecA* gene was amplified in *S. aureus* which showed no visible band size. The presence of coagulase negative staphylococcal DNA other than that associated with *S. epidermidis* was then detected by genus-specific probes.

Conclusion

Molecular and genotypic characterization of bacteria is advantageous when compared to biochemical and phenotypic methods of characterization and identification of nosocomial bacterial. The latter require a prolonged cultivation period for the suspected bacteria and pure bacterial cultures for various biochemical assays. It is therefore concluded that broad-range PCR amplification with subsequent hybridization on a microarray is a rapid diagnostic tool in identifying causative agents of bacterial infections in various specimens from normally sterile site of the hospital environment to medical and surgical tool surfaces.

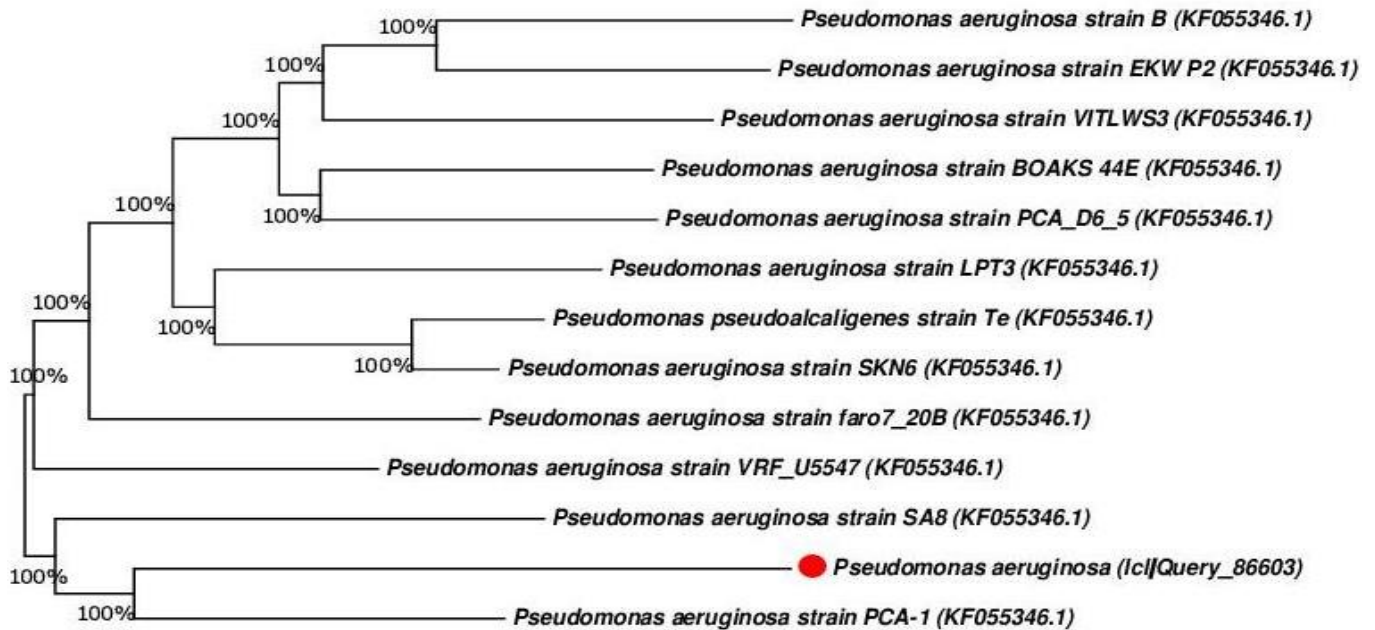


Figure 3. Phylogenetic tree of isolate with query id: 86603 Made by MEGA 7.0 showing percentage similarity of coding regions.

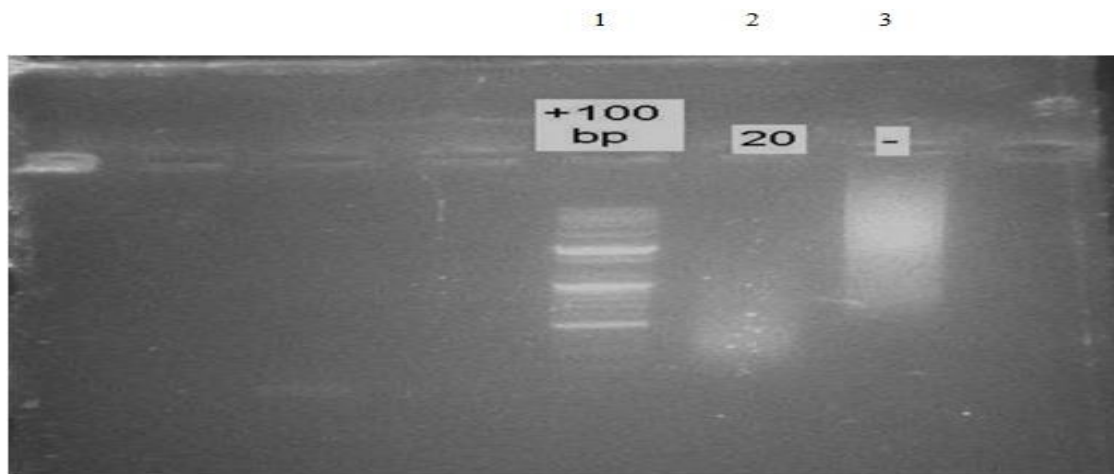


Figure 4. PCR amplification of *mecA* Gene in *S. aureus* showing no band. Lane 1, DNA marker; lane 2, *S. aureus*; lane 3, negative control.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

- Adeoye A (2007). Medical Laboratory Practice 1st edition, FEMCO Publishers Limited, Lagos, Nigeria, P 153.
- Agwung-Fobellah D, Kemajou ST (2007). Laboratory Microbiology and Activity Manual, Ark of Wisdom Publishers, Aba, Nigeria. pp. 12-37.
- Akihiro Ma, Yutaka T, Masami M (2017). Comparison of 16S ribosomal RNA gene sequence analysis and conventional culture in the

- environmental survey of a hospital. *J. Pharm. Health Care Sci.* 3(1):8.
- Ananthan G, Sivaperumal P, Hussain SM (2011). Antibacterial potential of marine ascidian *Phallusia Arabica* against isolated urinary tract infections bacterial pathogens. *Asian J. Anim. Sci.* 5(3):208-212.
- Ateba CN, Mbewe M (2011). Detection of *Escherichia coli* O157:H7 virulence genes in isolates from beef, pork, water, human and animal species in the Northwest Province, South Africa: Public health implications. *Res. Microbiol.* 162:240-248.
- Bergey's Manual of Determinative Bacteriology (1994). 9th edition, Holt, J.D. (Ed.), Williams Wilkins CO. Baltimore, P.783.
- Black JA, Foarde KK (2007). Comparison of four different methods for extraction of *Stachybotrys chartarum* spore DNA and verification by real-time PCR. *J. Microbiol. Methods* 70:75-81.
- Barrow GH, Feltham, RKA (1993). *Cowan and Steel's Manual for Identification of Medical bacteria*. Third edition. Cambridge University Press. Cambridge, Great Britain. pp. 245-247.
- Cheesbrough M (2006). *District laboratory practice in Tropical Countries Part 2* (5th Edition), Cambridge University Press. Cambridge. pp. 132-143.
- Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, Kulam-Syed-Mohideen AS, McGarrell DM (2009). The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Res.* 37:141-145.
- Daniel JD, Michael AP (2015). Prevention of health care-associated infections. In: James HJ, Michael AP, Karen CC, Guido F, Marie LL, Sandra SR, David WW, editors. *Manual of Clinical Microbiology*. 11th ed. Washington: American Society for Microbiology. pp. 106-119.
- Frickmann H, Dekker D, Boahen K, Acquah S, Sarpong N, Adu-Sarkodie Y (2013). Increased detection of invasive enteropathogenic bacteria in pre-incubated blood culture materials by real-time PCR in comparison with automated incubation in Sub-Saharan Africa. *Scand. J. Infect. Dis.* 45:616-622.
- Kerremans JJ, Verboom P, Stijnen T, Hakkaart-van Roijen L, Goessens W, Verbrugh HA, Vos MC (2008). Rapid identification and antimicrobial susceptibility testing reduce antibiotic use and accelerate pathogen-directed antibiotic use. *J. Antimicrob. Chemother.* 61:428-435.
- Kumar S, Stecher G, Tamura K (2016). MEGA7: Molecular Evolutionary Genetic Analysis Version 7.0. *Mol. Evol.* 33(7):1870-1874.
- Lee YA, Sung AN, Liu TF, Lee YS (2009). Combination of chromogenic differential medium and *estA*-specific PCR for isolation and detection of phytopathogenic *Xanthomonas* spp. *Appl. Environ. Microbiol.* 75:6831-6838.
- Ochei JO, Kolhatkar AA (2008). *Medical Laboratory Science: Theory and Practice*. Tata McGraw-Hill Publishing Company Limited, New York, pp. 311-347.
- Psifidi A, Dovas CI, Banos G (2010). A comparison of six methods for genomic DNA extraction suitable for PCR-based genotyping applications using ovine milk samples. *Mol. Cell Probes* 24:93-98.
- Smith K, Diggle MA, Clarke SC (2003). Comparison of commercial DNA extraction kits for extraction of bacterial genomic DNA from whole-blood samples. *J. Clin. Microbiol.* 41:2440-2443.
- Souza C, Faria YV, Sant'Anna LO, Viana VG, Seabra SH, Souza MC, Vieira VV, Hirata JR, Moreira LO, Mattos-Guaraldi AL (2015). Biofilm production by multiresistant *Corynebacterium striatum* associated with nosocomial outbreak. *Mem. Inst. Oswaldo Cruz.* 10(2):242-248.
- Yoshimura K, Morotomi N, Fukuda K, Nakano M, Kashimura M, Hachisuga T, Taniguchi H (2011). Intravaginal microbial flora by the 16S rRNA gene sequencing. *Am. J. Obstet. Gynecol.* 205(3):235-e1.