Antibacterial activity of some nano particles on antibiotic-resistant bacterial pathogens from the air of operation theatre

Abstract:

The current research work was carried out to find the antibacterial activity of some nano particles against bacterial pathogens isolated from the air of operation theatre of Mayo hospital, Lahore, Pakistan. Three pathogenic bacterial strains were isolated, namely A1, A2, A3. Molecular characterization, optimum growth conditions and antibiotic resistance of bacterial isolates were checked. The antibiotics used in this study were Amoxycillin**,** Cefepime and Ampicillin. Nano particles were used in methanolic solutions (mg/ml). Nano particles included ferric oxide, Zinc oxide and Silver Oxide. Results showed A3 was resistant to all antibiotics. Other strains showed sensitivity and resistance to these three antibiotics. All nano particles showed antibacterial activity against pathogenic bacterial isolates. Maximum zone of inhibition of 1cm was formed when used Ferric oxide against the A1 bacterial pathogen. Optimum temperature was 37ºC while the optimum pH was 7. These bacterial pathogens were identified by ribotyping as *Staphylococcus aureus* (A1), *Pseudomonas aeruginosa* (A2) and *Streptococcus pyogenes* (A3).

Keywords: bacterial pathogens; nano particles; antibacterial activity; ribotyping

Introduction:

Both pathogenic and non-pathogenic bacteria are present in the air. This contamination is increasing day by day due to increase in human population. Human population increase results in increased waste production, improper sanitary conditions and waste disposal problems (Hanif *et al*., 1995). Hospital indoor air contains a diverse group of micro-organisms. Here the significance of these microbes is put to the argument, whereas these may be considered significant in any other sphere. Farzana (1988) studied the airborne pathogenic bacterial isolates from various wards of Ganga Ram Hospital, Lahore. The work showed that the *Staphylococcus sp., Streptococcus pyogenes and Enterobacter sp.,* were frequent in hospital air. Airborne bacterial contamination in the operating theatre is one of the reasons for infections in connection with surgery. Because of overuse and misuse of antibiotics, the bacterial pathogens have become resistant and this resistance is increasing. So there is need

for additional therapies for infection control (Jaffal *et al.*, 1997). Nano particles are being used in research to study their antibacterial activity against these common pathogens. Nano particles range from 1 to 100 nm in size. Recent studies have proved that nano particles are not only effective in the treatment of cancer cells but also show significant antibacterial activity against common pathogens.

Materials and Methods:

Bacterial pathogens were isolated from the air of operation theatre. Sampling was done at specific selected points in the operation theatre. Random sampling was done to get better results. Sampling was conducted by exposing nutrient agar plates in operation theatre for three minutes. These plates were exposed at different points in operation theatre (Benson, 2002). After sampling, plates are placed in an incubator for overnight at 37^{o} C. Isolated bacterial colonies were streaked on fresh agar plated to obtain a pure culture. These pure cultures were subjected to blood agar test (following the methods of Khater & Elabd, 2016), antibiotic resistance/sensitivity test (following the methods of Nwankwo and Nasiru, 2011), nano particles resistance/sensitivity test (following the methods of Alaa El Dien et al, 2017), optimum growth conditions and molecular characterization (Cheesebrough, 1993).

Determination of Optimum Growth Conditions:

Optimum growth conditions for each bacterial isolate were determined. The optimum temperature of the three strains was observed. Optimum growth was studied at four different temperatures, 25ºC, 30ºC, 37ºC and 40ºC. The optimum pH of strains was also observed. The pH studied was 6.5, 7.0, 7.5 and 8.0.

Antibiotic resistance of bacterial pathogens:

Assessment of antibiotic resistance of bacterial pathogens was checked against broad-spectrum antibiotics by performing the disc diffusion method. For the test, nutrient agar plates were prepared for three strains. Bacteria were spread on the plates by spreading plate method. Antibiotics discs of known concentration were placed on the plates with the help of sterilized forceps and were incubated at 37 °C for 24 hours. Growth inhibited zones appeared as the clear area near the disc. Growth inhibited zones were measured. Clear zone indicated the sensitivity of tested bacterial strain against that antibiotic and no zone showed resistance.

Antibacterial activity test of Nano particles:

Antibacterial activity of various Nano particles was tested by well diffusion method (Buszewski et al 2018). The solution of Nano particles was made in the organic solvent *i.e.* Methanol The medium used was nutrient agar; it was prepared by dissolving 28 grams of prepared nutrient agar in 1 litre (1000ml) of distilled water in a flask. The pH of the medium was maintained at 7.4, the medium was sterilized by autoclaving for 20 minutes at 121^0C temperature and 15 Ib pressure. After the medium was autoclaved, it was poured in the Petri plates under sterile conditions, a drop of autoclaved water was poured in the centre of the plate on which bacterial isolate was inoculated and it was then evenly spread on the entire plate with the help of sterilized spreader. After that, wells were made in the plates. Solutions (1mg/ml) of three Nano particles i.e. Ferric oxide, Silver oxide and Zinc oxide were used. 50 micro liters solution of Nano particles were poured separately in the wells and 50 micro liters of methanol was also poured in a separate well as a control. Petri plates were covered with lids and incubated at 37^0C for 24 hours. After incubation, the zone of inhibition around the wells showed the sensitivity of the isolate against a particular particle whereas growth around the well indicated that the bacterial isolate was resistant against the particular particle.

Molecular characterization

Ribotyping or molecular characterization of 16s rRNA gene was done. Genomic DNA was isolated by phenol:chloroform extraction method. PCR was done using universal primers; 27f and 1495r (Bianciotto *et al.* 1996). After PCR gene clean was done and then sequencing from the molecular laboratory, Malaysia.

Results:

From air sample taken from operation theatre (Mayo hospital). Three bacterial pathogens A1, A2, A3 were identified as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes* by ribotyping. Bacterial pathogens showed resistance against antibiotics used. Bacterial strain A3 was most resistant against Amoxycillin**,** Cefepime and Ampicillin (Table 1). The sensitivity/resistance was checked by measuring Zone of inhibition. The zone of inhibition was measured in centimetre (cm).

Table 1: Antibiotic resistance/sensitivity of bacterial pathogens

strain	Amoxycillin (AMC 30ug)	Ampicillin (AMP 30ug)	Cefepime (CF 30ug)
	cm	cm	cm
A1	R	S (0.8)	S (0.4)
A2	R	R	S (0.7)
A3	R	R	R

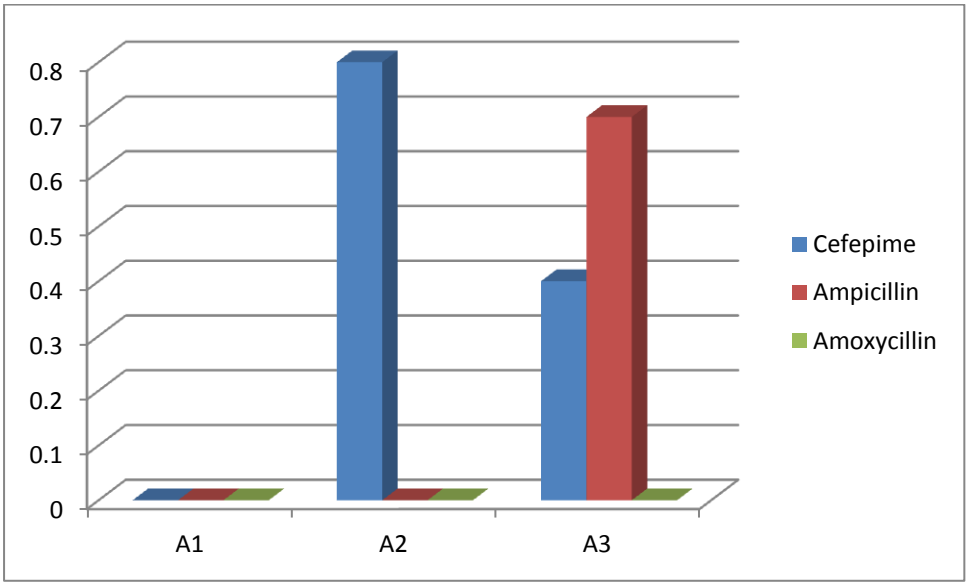

Figure 1: Antibiotic resistance/sensitivity of bacterial pathogens A1, A2 and A3

Antibacterial activity of nano particles was also studied. All bacterial pathogens were resistant against control solution of nano particles i.e, methanol. But nano particles showed clear antibacterial activity against all antibiotic-resistant bacterial pathogens (Table 2). Ferric oxide solution showed maximum antibacterial activity against A1(S*taphylococcus aureus*) by forming Zone of inhibition of 1cm while zinc oxide formed zone of inhibition of 0.3cm against A3(*Streptococcus pyogenes*).

Table 2: Antibacterial activity test of Nano particles

Nano particles solutions	Strain A1	Strain A2	Strain A3
Ferric oxide (1mg/ ml)	1.0cm	0.6cm	0.5cm
Zinc oxide (1mg/ml)	0.6cm	0.7cm	0.3cm
Silver oxide (1mg/ml)	0.9cm	0.9cm	0.6cm
Methanol (control)	R	R	R

R= RESISTANT

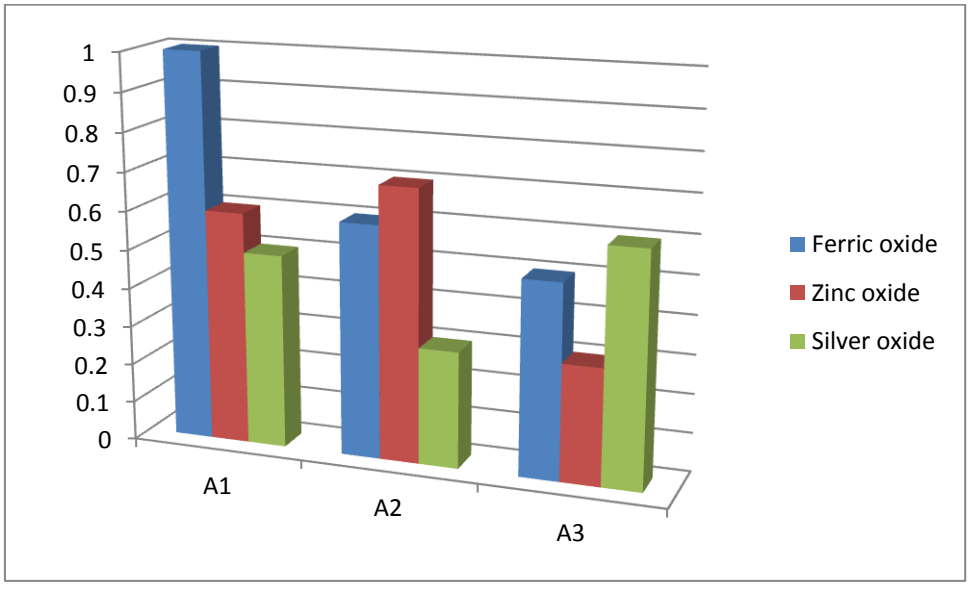

Figure 2: Antibacterial activity of nano particles against bacterial pathogens A1, A2 and A3

Optimum growth conditions were also observed. The optimum temperature for all strains was 37°C and that optimum pH was 7.

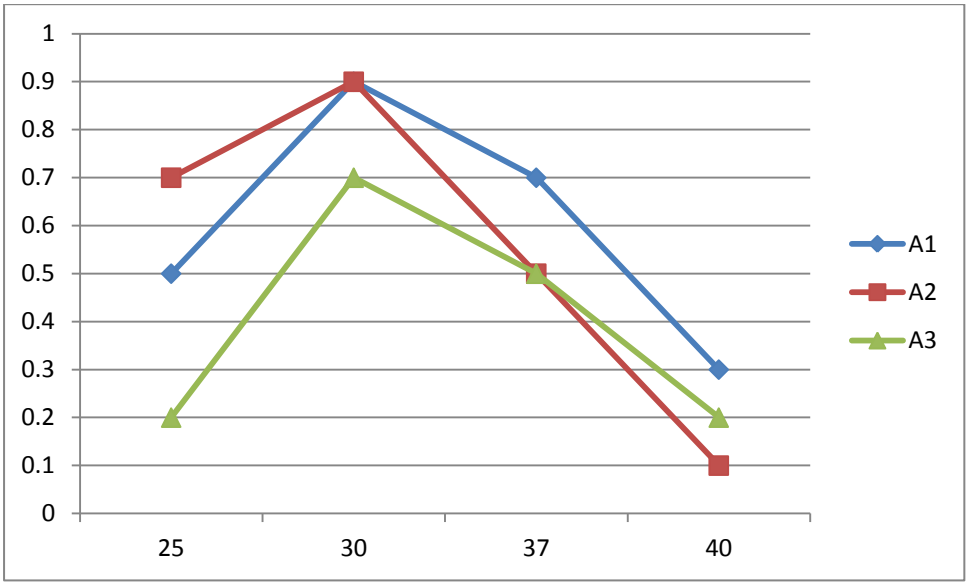

Figure 3: Optimum Temperature (°C) of bacterial pathogens

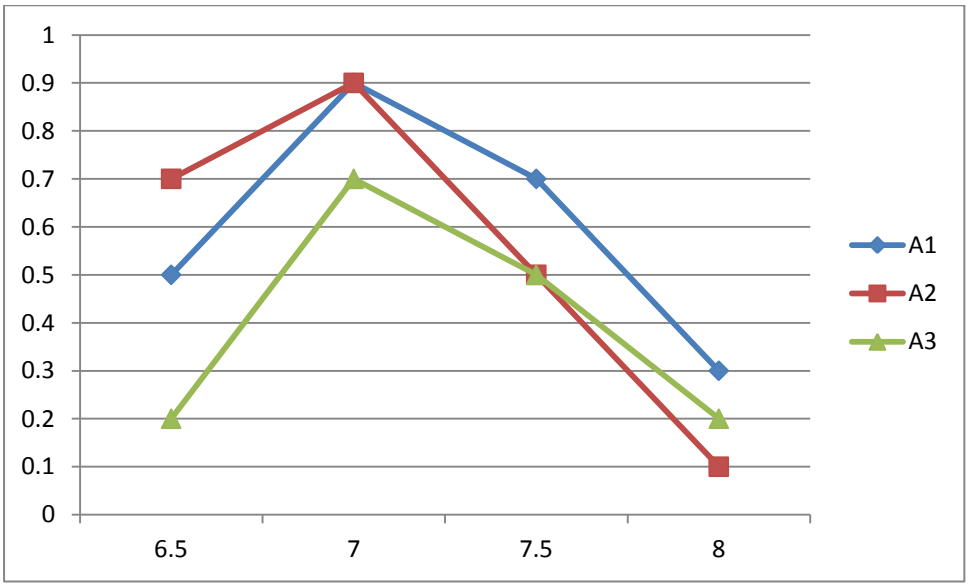

Figure 4: Optimum pH of bacterial pathogens

For molecular characterization sequences obtained were blast on NCBI website.

***Staphylococcus aureus* (partial sequence 16s rRNA gene)**

TTTATGGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAG
CGAACGGACGAGAGCTTGCTTCTATGATGTTAGCGGCGGACGGGTGAGTAACACGTGGATAACCT

ACCTATAAGACTGGGATAACTTCGGGAACCGGAGCTAATACCGGATAATATTTTGAACCGCATGG
TTCAAAAGTGAAAGACGGTCTTGCTGTCACTTATAGATGGATCCGCGCTGCATTAGCTAGTTGGTA
AGGTAAGTTACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGA
GACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGTCTTCCGCAATGGGCGAAAGCCTGACGG
CCGAGCAACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACA
TATGTGTAAGTAACTGTGCACATCTCGCGGTACCTAATCAGAAAG

Streptococcus pyogenes (partial sequence 16s rRNA gene)

GAGAGTTTGATCCTCCGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTAGAACGCT
GAGAACTGGACTTGCACCGGTTCAAGGAGTTGCGAACGGGTGAGTAACGCGTAGGTAACCTACCT
CATAACGGGGGATAACTATTGGAAACGATAGCTAATACCGCATAAGAGAGACTAACGCATGTTAG
TAATTATAAAAGGGGCAATTGCTCCACTATGAGATGGACCTGCGTTGTATTAGCTAGTTGGTGAGG
TAAAGGCTCACCAAGGCGACGATACATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGA
GACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGGGGCAACCCTGAC
CGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGAGAAGAATAG
GTGGGAGTGGAAAATCCACCAAGTGACGGTAACTAACCAGAAAGGGACG

Pseudomonas aeruginosa (partial sequence 16s rRNA gene)

GGTGCACAGCCGTCTGAGCGCGTTGCTCAGCTGCTCAAGGACGCTGCCAAGGCAAACGCCTAAGC
CGTCATGAGTGAAATGCCGACACCCGCCGACGACCTGGTCGTGATCGGCAAGATCGTTTCGGTGTA
CGGCATCCGCGGTGAGGTGAAGGTGTATTCCTTTACCGACCCGTTGGACAACCTGCTGGACTATCG
CCGCTGGACGCTCCGGCGCGACGGCGAGATTCGGCAGGCCGAGCTGGTCAGGGGGCGCCTGCATG
GCAAGGTCCTGGCCGCCAAGCTCAAGGGGCTCGACGATCGCGAAGAGGCCCGCACCTTCACCGGT
TACGAGATCTGCATCCCGCGTAGCGAGTTGCCCTCTCTCGAGGAAGGTGAGTACTACTGGCACCAG
CTGGAAGGCCTGAAGGTGATCGACCAGGGCAGGCAGTTGCTCGGCGTGATCGACCATCTGCTGGA
AACCGGTGCCAACGATGTCATGGTGGTCAAGCCCTGCGCGGGCAGCCTGGACGACCGCGAGCGCC
TGTTGCCCTACACCGGGCAGTGCGTGCTGTCGATCGACCTGGCCGCTGGCGAGATGCGGGTGGACT
GGGACGCGGACTTCTGATCATCCATGGACAAGCGTTTGTGGGTGGGCGTCGTCAGCATCTTTCCGG
AGATGTTCCGCGCGATCAGTGACTATGGCAT

Discussion:

In a recent study, bacterial pathogens were isolated from operation theatre (OT) air. The air of OTs is supposed to be sterile and bacteria free but countries like Pakistan where hygienic conditions are not ideal, contamination of air is an issue. So present work was carried out to study these common pathogens not only present outdoor but also in the indoor environment even places like OTs. The bacterial pathogens isolated are of common occurrence in hospitals yet their presence in the air of OT is questionable. Airborne bacterial pathogens introduced at surgery are an important source of wound contamination and joint sepsis. It has already been shown that even in ultraclean-air operating theatres; the surgical sucker forms a reservoir for those organisms which have been implicated in septic loosening of the prostheses (Whyte *et al*., 1991; Hanif *et al.,* 1995).

The bacterial strains isolated were *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa*. *Staphylococcus aureus* is most common pathogen among all in the environment and its infections are most common. *S. aureus* is Gram +ve cocci present in

form of clusters or bunches. It is coagulase positive which differentiates it from other species. *Streptococcus* sp. is Gram +ve cocci found in chains. Its infections are most common in operation wounds or postoperative wounds. *Pseudomonas aeruginosa* is commonly found in the air of hospitals or soil near to the hospitals. It is oxidase positive and is an opportunistic pathogen (Cheesebrough, 1993).

The present study also provided data related to the continuous increase in drug resistance against certain bacterial species. The misuse and overuse of antibiotics against infectious diseases result in the increase of drug resistance ability of microorganisms including bacteria (Canu *et al.*, 2002).

Nano particles are being extensively used to study antibacterial activity as these are considered as bactericidal agents. Many studies have shown that nano particles like a ferric oxide, zinc oxide and especially silver oxide are used as bactericidal agents. This property is because of their small size thus contributing to bactericidal activity. In a recent research study, the nano particles have shown significant antibacterial activity against locally isolated common bacterial pathogens. Almost all bacterial pathogens are antibiotic resistant yet shoed sensitivity against nano particles by forming clear zones. (Taylor and Webster, 2009). So in future, the nano particles are strong candidates of being bactericidal agents against drug/antibiotic resistant bacterial pathogens

Now there is a need to minimize or diminish the bacterial pathogens from OTs air as it is life-threatening. There is a need to improve sterile techniques and hygienic conditions, so that chances of operative or postoperative infections would be minimized.

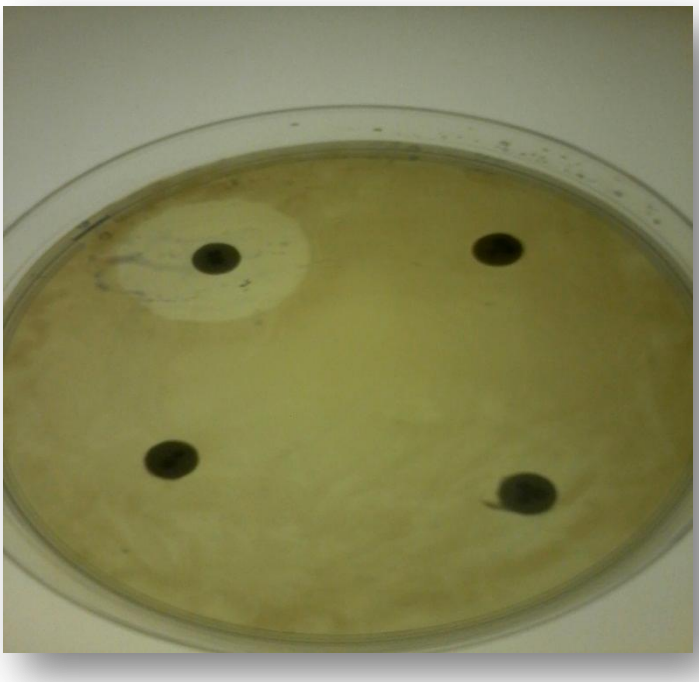

Figure 5: Antibiotic resistance/sensitivity test

Figure 6: Nano particles antibacterial activity test

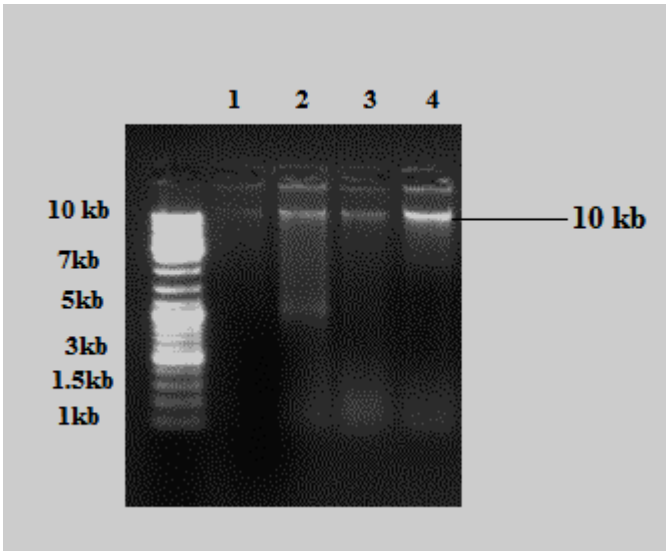

Figure 7: 1% agarose gel electrophoresis of bacterial genomic DNA

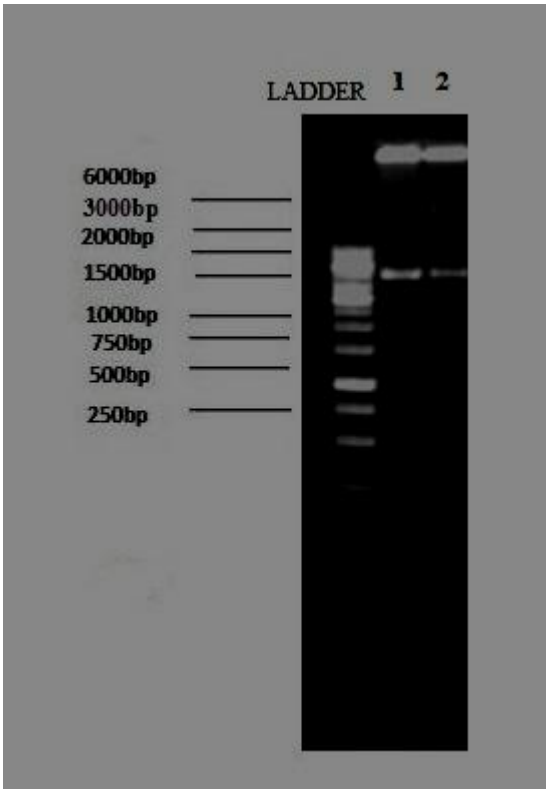

Figure 8: PCR product of bacterial strains after agarose gel electrophoresis

************Consent Disclaimer:**

N/A

Ethical Disclaimer:

As per international standard or university standard written ethical permission has been collected and preserved by the author(s).

Acknowledgement:

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References:

Canu, A. Malbruny, B. Coquemont, M. Davies, T.A. Appelbaum, P.C. and Leclercq, R. 2002. Diversity of ribosomal mutations conferring resistance to macrolides, clindamycin, streptogramin, and telithromycin in *Streptococcus pneumonia*. *Antimicrob. Agents Chemother.* **46**: 125–131.

Cheesebrough, M., 1993. Medical Microbiology; Manual for tropical countries, Vol. 2. Microbiology ELBS, University Press, Cambride.

Farzana,R., 1988. Studies on microbial pollution in the air of General Hospital, Lahore. M.Sc. Thesis, Department of zoology, University of the Punjab, Lahore.

Hanif, A., Jafri, R.H. and Baber, A., 1995. Studies on the prevalence of pathogenic bacteria in the air of Lahore. Punjab University. J.Zool. **10**:55-61.

Jaffal, A. A., Banat, I. M., EI Mogheth, A. A., Nsanze, H., Benar, A. and Ameen, A. S., 1997. Residential indoor airborne microbial populations in the United Arab Emirates. *Environ. Int.*, **23**(4):529-533.

Taylor, E.N. and Webster, T.J., 2009. The use of supra magnetic nano particles for prosthetic biofilm. *Int. Jr. Nanomed*. **4**:145-152.

Khater, W. S., & Elabd, S. H. (2016). Identification of Common Bacterial Pathogens Causing Meningitis in Culture-Negative Cerebrospinal Fluid Samples Using Real-Time Polymerase Chain Reaction. *International journal of microbiology*, *2016*, 4197187.

Nwankwo, E. O., & Nasiru, M. S. (2011). Antibiotic sensitivity pattern of Staphylococcus aureus from clinical isolates in a tertiary health institution in Kano, Northwestern Nigeria. *The Pan African medical journal*, *8*, 4.

Alaa El Dien M.S. Hosny, Marwa M.A, Rasha M.M. The antimicrobial effects of silver nanoparticles on the multidrug resistant klebsiella clinical isolates. Res. J. Pharm. 2017, 8 (9).

Bianciotto, V., Bandi, C., Minerdi, D., Sironi, M., Tichy, H.V. *and* Bonfante, P. *(*1996*)* An obligately endosymbiotic fungus itself harbors obligately intracellular bacteria. Appl Environ Microbiol 62, 3005–3010.

Buszewski, B. et al. Antimicrobial activity of biosilver nanoparticles produced by a novel Streptacidiphilus durhamensis strain, Journal of Microbiology, Immunology and Infection, Volume 51, Issue 1, 2018,Pages 45-54.