Evaluation of bactericidal potential of biological agents against strains of *Pseudomonas aeruginosa* from respiratory infections in neonates and infants

Running title: *Pseudomonas aeruginosa* and respiratory infections

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Abstract

The identification and testing of various pathogenic strains of *Pseudomonas aeruginosa* in the current study were conducted in the infection of the respiratory tract. Resistance and susceptibility of isolated strains were checked against different antibiotics, nanoparticles and by different plant extracts by using well diffusion method. Nanoparticles' antibacterial activity showed a significant difference as p-value was smaller than 0.05, indicating that they showed significantly better results as indicated by their inhibition zones. Against all pathogenic strains of *P.aeruginosa*, Pepal (*Ficus religiosa*), Eucalyptus (*Eucalyptus radiate*) and Clove (*Syzygium aromaticum*) silver nanoparticles exhibited magnificent results. The plant extracts and antibiotics didn't show any noticeable effect against pathogenic strains, as their p-values were greater than 0.05. This revealed that *P.aeruginosa* is developing resistance against various antibiotics. Further post hoc analysis followed by Bonferroni test revealed that Pepal (*Ficus religiosa*) exhibited relatively higher anti-bacterial effects compared to other plant extract. Eucalyptus (*Eucalyptus radiata*) and jamon (*Syzygium cumene*) have shown good anti-bacterial effects. All isolated strains showed sensitivity against Ciprofloxacin and Levofloxacin antibiotics as it was showed by creation of inhibition zone. Bacterial strains showed resistance against metronidazole as no zone of inhibition appeared. Pepal (*Ficus religiosa*) showed maximum inhibition zone with

a value of 20.5±0.20 mm by silver nanoparticle. Clove (*Syzygium aromaticum*) has shown antibacterial activity only against a few bacterial strains. The results revealed that *P. aeruginosa*, an opportunistic pathogen, is present in various infections in newborns' respiratory systems. Overuse and incorrect use of antibiotics have led to antibiotic resistance. To control the rise of resistance in *P aeruginosa*, it is recommended to switch antibiotics with other essential tools i.e. nanoparticles and plant extracts. Pepal (*Ficus religiosa*), Clove (*Syzygium aromaticum*) and Eucalyptus (*Eucalyptus radiata*) have remarkable properties in their silver nanoparticles, and they can be used as anti-bacterial agents against *P. aeruginosa.*

Key words: Pathogenic strains, Respiratory tract infections, Pathogenicity, Beta hemolysis, Antibiotics, Nanoparticles, Plant extracts, well diffusion method.

INTRODUCTION

Bacteria are one of the charismatic microbes that play an important role in everyday life. Bacteria are usually known as the oldest creature on earth. Bacteria are prokaryotic, single-celled microbes having a number of species from which many are not described yet[1)]. The exceptional characteristics of bacteria empower them to live in extreme conditions and they can survive everywhere even in the intestine of human or in deep ocean[2)].

A number of bacteria are disease causing microbes while some are beneficial for human health and for ecosystem, like some bacteria are important for good gut health in humans and some bacteria fix environmental nitrogen[3,4)]. A technique known as Gram staining is used to differentiate between two categorize of bacteria whether the bacteria is gram negative or gram positive, as gram negative bacteria hold a pink color and gram positive hold a blue color after staining process.

On the basis of shape, gram negative rod shaped bacteria are bacilli, and *P. aeruginosa, Klebsiella, Escherichia coli, Citrobactor, and Salmonella,* included in gram negative bacteria. Some of them are lethal and cause major infections in humans including Respiratory tract infections (RTIs), Surgical site infections (SSIs), Urinary tract infections (UTIs) and Bloodstream infections (BSIs) [5-7)].

Respiratory system of human's body play a vital role in defense mechanism against microbial infection due to presence of mucus membranes and cilia small hair like

structures, both mucus membrane and cilia together with trap the airborne microbes and protect from infection[8,9].

Production of mucus and presence of cilia become the problem for microbes in the lower respiratory tract, because mucus trap the microbes and any foreign particle get trapped in mucus and cilia eliminate them from the tract by their movement[10-12]. Cells of defense mechanism in respiratory tract identify the microbe and protect the body from their invasion. Gram negative bacteria become the reason of different respiratory infections which can lead from curable to life threatening[13].

The bacterium *P. aeruginosa* can tolerate different conditions. The genome (5.5-7 Mbp) is relatively large compared to other sequenced bacteria such as *Bacillus subtilis* (4.2 Mbp), *E.coli* (4.6 Mbp), *Mycobacterium tuberculosis* (4.4 Mbp), and encodes a variety of regulatory enzymes that Involved in metabolism, flow and transport. The *P. aeruginosa* genome allows to cope with environmental changes, metabolic plasticity, and stressful conditions[14-17].

P. aeruginosa is an opportunistic pathogen that cause infection in immunocompromised persons and nosocomial infections and respiratory distress syndrome (RDS) and ventilator-associated pneumonia (VAP) are commonly caused by P. aeruginosa in infants and even in adults. *P. aeruginosa* can survive and multiple in humid environment and due to this quality it can easily adhere in equipment's used in hospitals and cause serious infections. Different nosocomial infections such as chronic obstructive pulmonary disease, cystic fibrosis, chronic obstructive pulmonary disease (COPD), bronchiectasis and RDS and due to its resistance, this pathogen is becoming dangerous to patients suffering from infections[18].

Different chemicals are naturally present in different plant extracts which act as an antibacterial agent for different infections. Bioactive substances are present in plant extracts which have antibacterial properties and have bacteriostatic and bactericidal effect. Plant extracts contain different components of defense system of plants such as phenolic chemicals like flavonoids and tannins, terpenoids, alkaloids and different secondary metabolites and some of them act as antibacterial agent[19, 20].

Nanoparticles have different peculiar physical and chemical properties for their antibacterial properties. This feature has significantly contributed to the creation of

nanoparticles for various applications, including medical devices, wound dressing, and drug delivery systems. There are many important factors that enhance the antibacterial activity of nanoparticles. Nano particles have a much larger volume-to-surface area ratio in most cases, which leads to more interactions with bacterial cells due to their expanded surface area[21-25)].

Due to negatively charged cell membrane of bacteria, nanoparticles actively attract the bacterial cell membrane and damage it due to which cell death of bacteria occur. Silver nanoparticles have great antimicrobial effect and due to this reason are commonly used for antibacterial activity. Different types of nanoparticles, such as silver, zinc oxide, titanium dioxide, and copper, have demonstrated strong antibacterial abilities[26,27)].

Materials and Methods

Patients' Approval:

The samples were taken by qualified pulmonologists and patients had no objection in giving sample. The study was approved by Board of studies (BOS), department of Zoology and Advance Studies and Research Board, Government College University Lahore (REG-ACAD-ASRB/57/24/021). Also approved by ethical committee of institution.

Sample Collection:

Samples of Nasal swabs of infants suffering from respiratory associated distress syndrome (RDS) such as pneumonia, were collected from Services Hospital, Lahore, Fatima Memorial Hospital, Lahore and Rashid Latif Medical College, Lahore. For transportation from hospital to Government College University Lahore the samples were stored and saved in insulated ice bags. Samples were immediately kept at 4°C and then microbiological examinations were carried out.

Isolation of Bacteria:

In order to isolate bacteria, Firstly, MacConkey agar media was prepared and for this purpose 10g of MacConkey agar was mixed in 50ml of distilled water and then prepared MacConkey agar media up to 200ml by adding distilled water up to the mark. Sterilization of media was done in autoclave. After sterilization, next steps were done in Air Laminar Flow, plates were made by pouring media into petri dishes and plates were kept for 10 minutes in order to allow the agar to get solidify. As the media settled down in plates, sample were directly spread on MacConkey agar media and after few

minutes plates were placed in incubator at 37ºC for 24 hours. Next day, after completion of incubation time period observation of bacterial colonies were done[28, 29] (Fig 1).

Isolation of Pure culture:

After observation of bacterial colonies on MacConkey agar, next step was to isolate bacterial colonies to prepare pure culture. For this purpose, Cetrimide agar media was prepared and for this purpose 9.34g of Cetrimide agar was dissolved in 50ml of distilled water and then prepared the Cetrimide agar media up to 200ml by adding distilled water up to the mark. Sterilization of media was done in autoclave. After sterilization, next steps were done in Air Laminar Flow, and the cetrimide media was poured into petri plates and allow the media to solidify in plates. After solidification of cetrimide media plates, bacterial colony from MacConkey agar media was picked by inoculating loop and streaking of colony on Cetrimide agar plates was done. Inoculating loop was sterilized before picking up colonies. After streaking of colonies Plated were incubated for overnight at 37ºC. After incubation, colonies of *Pseudomonas aeruginosa*, a pure culture of bacteria was observed under Ultraviolet light[30] (Fig 5).

Pathogenicity Test:

For isolation of pathogenic strains of *P. aeruginosa* pathogenicity test was done. For this test non-coagulated blood was mixed with nutrient agar media so also called as Blood Agar Test. For this purpose, 15gram of nutrient agar was mixed in 200ml distilled water and then make the solution up to the mark 500ml by adding distilled water and media was mix well. The media in conical flask was sterilized by using autoclave. After sterilization, few minutes later as media gets cool down or come to at normal temperature about 10ml of non-coagulated blood was mixed in media to prepare Blood agar media. Blood agar media was poured in petri plates under the laminar air flow cabinet and let the media settled down. After solidification of media colonies from pure culture were picked up by sterilized inoculating loop and streaked on blood agar media. After streaking plates were incubated for 24 hours at 37ºC. After incubation period, type of hemolysis shown by pathogen was checked[31-33] (Fig 6).

Preparation of inoculums:

After Inoculum preparation, 300ml of nutrient broth media was prepared and for this reason 9.9gram of nutrient broth powder was mixed in 100ml of distilled water and mixed well and then distilled water was added up to the mark of 300ml and properly mixed the media in water. Broth media was then poured in falcon tubes and sterilized in autoclave. Under laminar air flow by using sterilized inoculating loop single bacterial colony from pure culture was picked up and inoculated in broth media and placed the tubes in incubator for overnight at 37^{o}C[34)].

Glycerol stock preparation:

For the preparation Glycerol stock solution, laminar air flow cabinet was used and by using micropipette 800µl of broth culture was mixed with 200µl glycerol in sterilized Eppendorf. Each Eppendorf was containing the different broth culture. After preparation, glycerol stocks with bacterial strains were refrigerated for storage at 20^{o}C[35)].

Antibacterial activity test of various antibiotics:

To check antibacterial activity of different antibiotics, nutrient agar medium plates were prepared and for this 9.00gram of nutrient agar was dissolved in 100ml of distilled water and make the solution up to the mark of 300ml and sterilize the media in autoclave. After sterilization, media was poured in petri plates under laminar air flow cabinet and allow the media to stabilize.

Broth culture of bacteria were poured on media plates and spread uniformly by using sterilized glass spreader. Antibacterial activity was checked by well diffusion method, for this method wells were made in agar media in petri plates. For creation of wells, 100 µl sterilized tip was used and cut the media from wider side of tip into 5 parts. These parts were removed from the media plate by using sterilized forceps and by this method five wells were created. Four wells were used for antibiotics and fifth was used as a control in the central position of plate[36, 37)].

Solutions of different four antibiotics such as Ciprofloxacin, Azithromycin, Levofloxacin and Metronidazole was prepared by adding powdered antibiotic of 250mg into distilled water and make solution up to the level of 50ml. The antibiotic

solution of each antibiotic was loaded in four wells in quantity of 50 µl in well A, B, C and D by using micropipette respectively. Distilled water was added in well E as a control.

Plates were then incubated for overnight at 37°C. After completion of incubation time, zone of inhibition was observed to check whether the bacteria susceptible or resistant to specific antibiotic. Zone of inhibition was measured to check the susceptibility of bacteria to antibiotic.

Test of Nanoparticles' antimicrobial activity:

For preparation of different silver nanoparticles 1mM solution of silver nitrate was prepared and for this 0.018gram silver nitrate was dissolve in 100ml of deionized water. Eight different silver nanoparticles were used and for their preparation 1mM solution of silver nitrate was poured in different flasks dropwise containing different plant extracts such as Clove (*Syzygium aromaticum*), Neem (*Azadirachta indica*), Eucalyptus (*Eucalyptus radiata*), Onion (*Alium cepa*), Aleovera (*Aloe barbadensis*), Lemon (*Citrus limon*), Jamun (*Syzygium cumini*) and Pepal (*Ficus religiosa*), silver nitrate solution was added in flasks in 90:10 ratio. Mixture was then placed under sunlight till time they color of the mixture changed to brown or dark brown. As, the color of mixture was changed, flasks were covered with Aluminum foil and left for 24 hours. After 4 hours these eight different nanoparticles were used for antibacterial activity.

300ml of nutrient agar media was prepared and for this reason 9.9gram of nutrient agar was mixed in 100ml of distilled water and mixed well and then distilled water was added up to the mark of 300ml and sterilize the media in autoclave. After sterilization, as the media become normal to room temperature, poured into petri plates under laminar air flow cabinet and let the media to solidify[38-40)].

Broth culture of bacteria were poured on media plates and spread uniformly by using sterilized glass spreader. Antibacterial activity was checked by well diffusion method, for this method wells were made in agar media in petri plates. More than one plates were used for different isolates. For creation of wells, 100 µl sterilized tip was used and cut the media from wider side of tip into 5 parts. These parts were removed from the media plate by using sterilized forceps and by this method five wells were created. Four wells were used for silver nanoparticles and fifth was used as a control in the

central position of plate.

By using micropipette 50µl nanoparticles was loaded in wells and incubate the plates for overnight at 37°C. After completion of incubation time, zone of inhibition were observed to check whether the bacteria susceptible or resistant to specific nanoparticles. Zone of inhibition was measured to check the susceptibility of bacteria to nanoparticle[41)].

Antibacterial activity test of Plant extracts:

Different eight plant extract such as Clove (*Syzygium aromaticum*), Neem (*Azadirachta indica*), Eucalyptus (*Eucalyptus radiata*), Onion (*Alium cepa*), Aleovera (*Aloe barbadensis*), Lemon (*Citrus limon*), Jamun (*Syzygium cumini*) and Pepal (*Ficus religiosa*) were used to check antibacterial activity of extract against bacterial strains.

Leaves were crushed and dipped in distilled water in a beaker and beaker was heated on hot plate for 1-2 hours at 60°C. After completion of heating time, let the mixture cool down and filtrate was obtained in a flask by using filter paper. This filtrate was plant extract.

Onion (*Alium cepa*) and Aleovera (*Aloe barbadensis*) were cut into pieces and Clove (*Syzygium aromaticum*), along with slices of onion and aleovera dipped in distilled water in a beaker and heated on hot plate for few hours at 60°C. After completion of heating time, let the mixture cool down and filtrate was obtained in a flask by using filter paper. This filtrate was extract of onion, aleovera and clove.

300ml of nutrient agar media was prepared and for this reason 9.9gram of nutrient agar was mixed in 100ml of distilled water and mixed well and then distilled water was added up to the mark of 300ml and sterilize the media in autoclave. After sterilization, as the media become normal to room temperature, poured into petri plates under laminar air flow cabinet and let the media to solidify[42, 43)].

Broth culture of bacteria were poured on media plates and spread uniformly by using sterilized glass spreader. Antibacterial activity was checked by well diffusion method, for this method wells were made in agar media in petri plates. More than one plates were used for different isolates. For creation of wells, 100 µl sterilized tip was used and cut the media from wider side of tip into 5 parts. These parts were removed from the media plate by using sterilized forceps and by this method five wells were created.

Four wells were used for plant extracts and fifth was used as a control in the central position of plate.

By using micropipette 50µl plant extract was loaded in wells and incubate the plates for overnight at 37°C. After completion of incubation time, zone of inhibition were observed to check whether the bacteria susceptible or resistant to specific plant extract. Zone of inhibition was measured to check the susceptibility of bacteria to plant extract[44-46].

Results

Blood agar test of isolated strains

Five out of all the different types separately expressed hemolysis β (Table 1).

Antibiotic resistance of isolated bacterial strains ± S.E value

The zone inhibition values against ciprofloxacin were 39.6 ± 0.23, 38.3 ± 0.62, 41.0 ± 0.40, 49.0 ± 0.23, and 48.6 ± 0.62, while for levofloxacin they were 40.0 ± 0.40, 40.0 ± 0.40, 36.0 ± 0.40, 51.0 ± 0.23, and 50.0 ± 0.23 for strains A,B,C,D & E. The values of Zone of inhibition of strains A,B, C against azithromycin were 29.0 ± 0.40, 34.0 ± 0.23, and 33.6 ± 0.23, respectively, while D and E showed normal results against it. Strains exhibited resistance against metronidazole, and D and E showed resistance against azithromycin (Fig 7).

It's clear from the results in a way that there wasn't any significant difference in antibiotic dilutions, because the efficacy was 0.99, which is higher than 0.05. It shows that there was no difference between antibiotics and anti-bacterial resistance among all types. Further post-hoc analysis was done through t-test, which revealed no difference among different groups (Table 2).

Antibacterial test of Nano Particles ± S.E. value

The zone inhibition values against (*Eucalyptus radiata)* Eu. AgNP's were 14.5 ± 0.20, 14.6 ± 0.31, 14.8 ± 0.23, 10.1 ± 0.31 and 15.2 ± 0.27 for strains A, B, C, D and E respectively (Table 4).

The zone inhibition values against Clove AgNP's were 13.3 ± 0.23, 12.1 ±0.11 and 14.8 ± 0.31 for strains A, B, and C respectively. Strain D and E shown minor results against Clove AgNP's (Fig 3, 8).

The zone inhibition values against Onion, Neem, Alovera and lemon AgNP's were 15.4 ± 0.14, 12.8 ± 0.11, 14.0 ± 0.40 and 9.6 ± 0.16 for strain A respectively. The zone inhibition values against Onion, Neem, Alovera and lemon AgNP's were 14.0 ± 0.20, 14.1 ± 0.11, 9.7 ± 0.10 and 9.8 ± 0.11 for strain C respectively. Strain B, D and E shown minor results against Onion, Neem, Alovera and lemon AgNP's.

One way ANOVA indicated that there is a significant difference between various types of AgNP's is because the p-value was 0.0042, which is smaller than 0.05. Post-hoc by Tukey test indicated that there was a significant difference between B vs D and E vs D. Against all strains *Eucalyptus radiata* silver nano particles and *Ficus religiosa* silver nano particles indicated significant results. Antibacterial activity against strains A, B and C best shown by clove silver nano. Antibacterial activity against strains A and B best shown by silver nano of Onion, Neem, Alovera and Lemon. Minor results were shown against strains C. D and E. Antibacterial activity of Jamun silver nano showed no result against any strain.

Antibacterial test of Plant Extracts ± S.E value

The zone inhibition values against (*Ficus religiosa*) plant extract were 19.6 ± 0.62, 19.0 ± 0.40, 18.0 ± 0.40, 17.16 ± 0.51 and 29.16 ± 0.42 for strains A, B, C, D and E respectively (Fig 2, 9).

The zone inhibition values against (*Eucalyptus radiata)* Eu. plant extract were 15.5 ±0.20, 15.83 ± 0.42, 20.83 ± 0.31, 15.0 ± 0.20, 16.83 ± 0.51 for strains A,B,C,D and E respectively.

The zone inhibition values against (*Syzygium cumini*) Sc. Plant extract were 15.0 ± 0.20, 18.5 ± 0.20, 17.83 ± 0.31, 13.66 ± 0.62 for strains A, B, C, D respectively.Against Sc. plant extract minore result showed by strain D.

Neem or *Azadirachta indica* (Ai.) showed minor effects on strain A, B, D and E. Zone of inhibition ± S.E of Ai. Plant extract against C was 11.0 ± 0.40.

The zone inhibition values against Neem or *Azadirachta indica* (A.) was 11.0 ± 0.40 for strain C. All other strains A, B, D and E have minor effect against Neem

or *Azadirachta indica* (Ai). Against Clove, Aleovera, Onion and Lemon plant extract, minor rsults have been shown by all strains. It's clear from the results in a way that there wasn't any significant difference in antibiotic dilutions, because the efficacy was 0.94, which is higher than 0.05. It shows that there ws no difference between anti-biotics and anti-bacterial resistance among all types. Further post-hoc analysis was done through t-test, which revealed no difference among different groups (Table 3).

Strains	**Pathogenicity**	**Hemolysis**
Strain A	+ve	β-hemolysis
Strain B	+ve	β-hemolysis
Strain C	+ve	β-hemolysis
Strain D	+ve	β-hemolysis
Strain E	+ve	β-hemolysis

Table. 1 Blood agar test of isolated strains

Strains	Metronidazole	Ciprofloxacin	Levofloxacin	Azithromycin
Strain A	1 ± 0	39.6 ±0.23	40.0 ± 0.40	29.0 ± 0.40
Strain B	1 ± 0	38.3 ± 0.62	40.0 ± 0.40	34.0 ± 0.23
Strain C	1 ± 1.33	41.0 ± 0.40	36.0 ± 0.40	33.6 ± 0.23
Strain D	2 ± 0	49.0 ± 0.23	51.0 ± 0.23	1 ± 1.33
Strain E	2 ± 1.33	48.6 ± 0.62	50.0 ± 0.23	2 ± 0

Table. 2 Antibiotic resistance of isolated bacterial strains ± S.E value.

Strains	Strain A	Strain B	Strain C	Strain D	Strain E
Clove	1 ± 0	1 ± 1.33	1 ± 0	2 ± 1.66	2 ± 1.33
Pepal	19.6 ± 0.62	19.0 ± 0.40	18.0 ± 0.40	17.16 ± 0.51	29.16 ± 0.42
Aleovera	1 ± 0	1 ± 0	1 ± 1.33	1 ± 1.33	1 ± 0
Onion	1 ± 1.33	2 ± 1.66	1 ± 1.33	2 ± 1.33	1 ± 0

Neem	1 ± 0	1 ± 0	11.0 ± 0.40	1 ± 1.33	1 ± 0
Eukalyptus	15.5 ±0.20,	15.83 ± 0.42,	20.83 ± 0.31,	15.0 ± 0.20,	16.83 ± 0.51
Lemon	1 ± 1.33	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Jamun	15.0 ± 0.20	18.5 ± 0.20	17.83 ± 0.31	13.66 ± 0.62	2 ± 1.66

Table. 3 Antibacterial test of Plant Extracts

Strains	Strain A	Strain B	Strain C	Strain D	Strain E
Clove AgNP's	13.3 ± 0.23	12.1 ± 0.11	14.8 ± 0.31	1 ± 0	1 ± 0
Pepal AgNP's	20.5 ± 0.20	14.33 ± 0.8	14.3 ± 0.23	10.1 ± 0.11	15.1 ± 0.11
Alovera AgNP's	14.0 ± 0.40	1 ± 1.33	9.7 ± 0.10	2 ± 1.33	2 ± 1.33
Onion AgNP's	15.4 ± 0.14	2 ± 1.33	14.0 ± 0.20	2 ± 1.66	2 ± 1.33
Neem AgNP's	12.8 ± 0.11	1 ± 0	14.1 ± 0.11	1 ± 1.33	1 ± 1.33
Eukalyptus AgNP's	14.5 ± 0.20	14.6 ± 0.31	14.8 ± 0.23	10.1 ± 0.31	15.2 ± 0.27
Lemon AgNP's	9.6 ± 0.16	2 ± 1.33	9.6 ± 0.11	1 ± 1.33	1 ± 1.33

Jamun AgNP's	1 ± 1.33	1 ± 0	1 ± 1.33	1 ± 1.33	1 ± 1.33

Table. 4 Antibacterial effect of Nano particles ± S.E

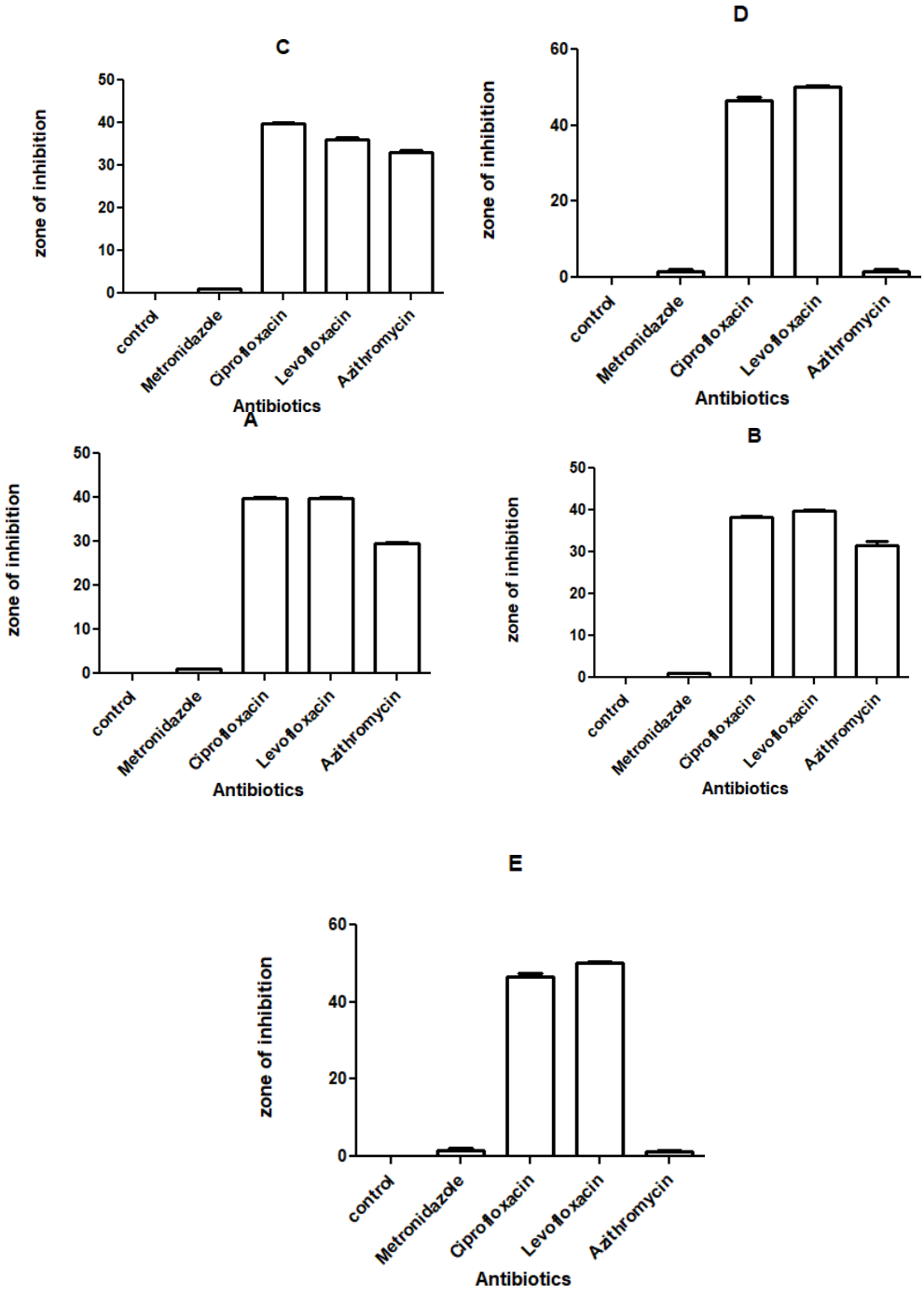

Fig. 1 Effect of Antibiotics on isolated strains

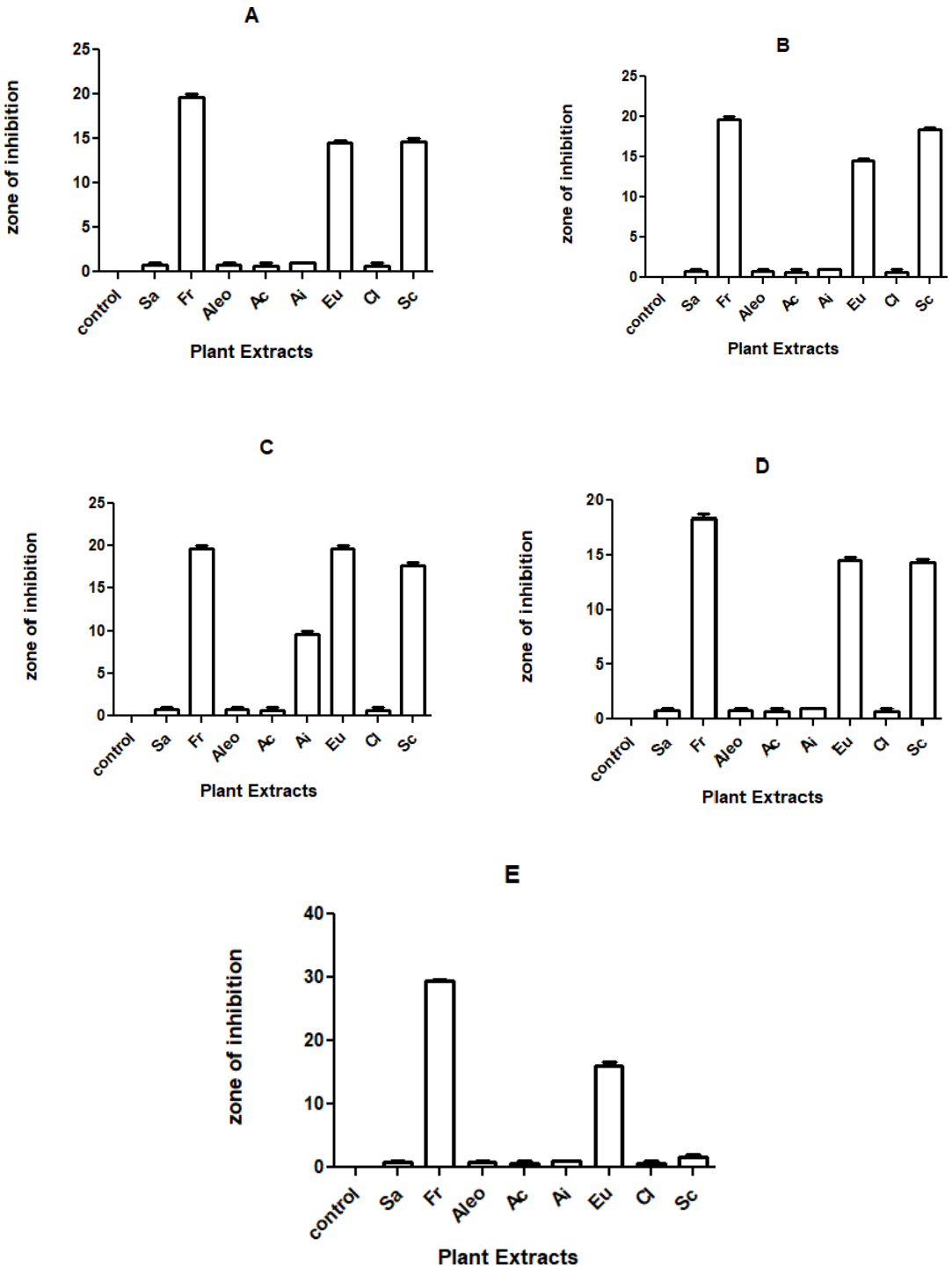

Fig. 2 Effect of Plant extracts on isolated strains

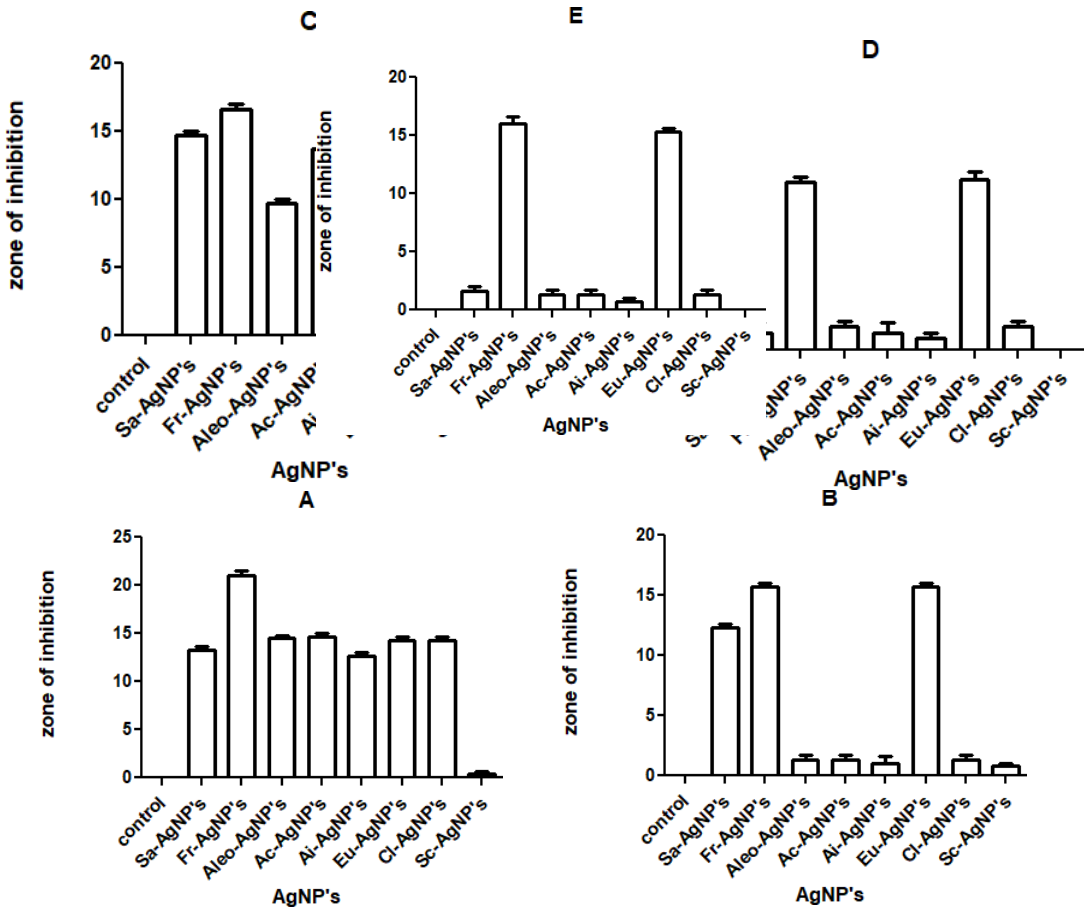

Fig. 3 Effect of AgNP's on isolated strains

Fig. 4 Streaking Plates of bacterial strains on MacConkey Agar

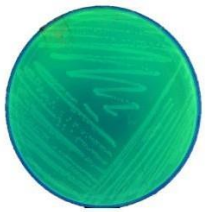

Fig. 5 Streaking of Pure culture of *P.aeruginosa* on Cetrimide agar

Fig. 6 Blood agar or pathogenicity test of isolated bacterial strains

Fig. 7 Effect of antibiotics on isolated bacterial strains

Fig. 8 Antibacterial activity test of Nanoparticles

Ai, Eu Sc, Fr are abberivated for Neem (*Azadirachta indica*), Eucalyptus (*Eucalyptus radiata*), Jamun (*Syzygium cumini*) and Pepal (*Ficus religiosa)* respectively

Fig. 9 Antibacterial activity test of Plant extracts. (Ai, Fr and Eu are abberivated for Neem (*Azadirachta indica*), Eucalyptus (*Eucalyptus radiata*), and Pepal (*Ficus religiosa)* respectively).

Discussion

The outcomes of the present study that none of the bacterial strains showed resistance to Ciprofloxacin antibiotic. This result was confirmed by previous studies as Ciprofloxacin is an efficacious antibiotic in the treatment of lung infections especially caused by *P. aeruginosa*[47].

All bacterial strains in the current study also showed susceptibility to Levofloxacin

antibiotic. The result were confirmed by preliminary studies because Levofloxacin is a broad-spectrum antibiotic and is very effectual against formation of biofilms produced by *P.aeruginosa* (Razdan, K. *et al*., 2023). Another study confirmed that Levofloxacin is the strongest antibacterial agent against P.aeruginosa species and able to kill all *P.aeruginosa* strains[48,49)].

Two out of five bacterial strains showed resistance against Azithromycin which point out that bacteria are now developing resistance against azithromycin. These findings were confirmed by previous research showing that *P.aeruginosa* is now becoming resistant to various drugs, inclusive of Azithromycin, therefore, combination therapy is effectual against *P.aeruginosa*.

All bacterial isolates in current study showed resistance against Metronidazole. This result was confirmed by past studies that concluded that 20-230% bacterial strains have resistance against metronidazole[50)].

The result of current study concluded that extracts of different plants such as Pepal (*Ficus religiosa*), Eucalyptus (*Eucalyptus radiata*) and Jamun (*Syzygium cumini*) are great antibacterial agent. The results were confirmed by preliminary studies that proved the presence of a high amount of flavonoid in the extract of eucalyptus, which increases its antibacterial activity. Another study confirmed that eucalyptus extract has shown its antibacterial activity against a very wide range of microbes causing respiratory infections, including *P.aeruginosa.*

Results from previous work confirmed that Jamun (*Syzygium cumini*) has very strong antioxidant and antibacterial properties and it's showed by strong inhibition zones against bacterial strains, confirming its antibacterial properties and indicates that it would be a potential therapy for the treatment of pathogens[51-54)].

Another previous research study concluded that Phytochemicals are present in *Syzygium cumini* which are used for manufacturing of drugs used in microbial infections. Different ayurvedic medicines are also manufactured by leaves of *Syzygium cumini.* Jamun contains gallic acid, mallic acid, essential oils, flavonoids which are important for antibacterial activity of Jamun.

In the current study Silver nanoparticles of Clove (Syzygium aromaticum), Eucalyptus

(Eucalyptus radiata) and Pepal (Ficus religiosa) indicated antibacterial activity to a maximum level. The past studies validated this result that silver nanoparticles of Clove (*Syzygium aromaticum*) revealed maximum antibacterial properties[55,56]. Reactive oxidative species (ROS) and free radicals are produced by *Syzygium aromaticum* species that inhibit the microbial activity in lung infection.

Eucalyptus (*Eucalyptus radiata*) silver nanoparticles have anti-biofilm activity against Gram-negative microorganisms at concentrations of 62.5 µl and 125 µl which showed its effectiveness against the multi-drug resistant property microbes, making it an environmentally friendly tool in nanomedicine formulation.

Previous study on the antibacterial potential of Pepal (*Ficus religiosa*) concluded that pepal contains natural compounds such as antioxidants and phytochemicals due to which it become effectual antimicrobial agent, but antibacterial activity of *Ficus religiosa* against respiratory tract infections, especially *P.aeruginosa*, was not evaluated, which this study showed could be a potential treatment[57-60].

The current study concludes that due to overuse and misuse of antibiotics *P.aeruginosa* is becoming resistant to different antibiotics. Clove (*Syzygium aromaticum*), Eucalyptus (*Eucalyptus radiata*) and Pepal (*Ficus religiosa*) silver nanoparticles can an effectual in treatment against respiratory infections caused by *P.aeruginosa* and in the formulation of nanomedicines. Since they are made by green synthesis, they do not cause pollution. This study also showed that papal (*Ficus religiosa*), eucalyptus (*Eucalyptus radiata*) and jamon (*Syzygium cumini*) have antibacterial properties in their plant sap, which can be used for the treatment of virulent pathogen *P. aeruginosa*.

Consent for publication

There is no conflict of interest among authors

Availability of data and material

Available

Conflict of Interest

No competing interests among authors

Funding

None

Ethical Approval

The study was approved by Board of studies (BOS), department of Zoology and Advance Studies and Research Board, Government College University Lahore (REG-ACAD-ASRB/57/24/021).

Patients' approval

There was no research work on humans. Only sputum samples were collected from expert doctors who took samples with consent of patients.

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