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Antibacterial efficacy of silver nanoparticles against metallo-β-lactamase (*bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA}$) producing clinically isolated *Pseudomonas aeruginosa*

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Abstract: Carbapenem resistance in *Pseudomonas aeruginosa* is a major concern in the public health sector, primarily in developing countries such as Pakistan. Therefore, novel approaches such as Silver nanoparticles (AgNPs) can be used to address emerging concerns. Clinical isolates (n=200) were reconfirmed using selective media and API 20NE kit. The antibiogram was determined according to the CLSI 2016 guidelines. Molecular detection was carried out by PCR. Antibacterial activity in AgNPs was achieved by dilution method. Of 200 *P. aeruginosa*, mostly (n=82; 41%) were isolated from pus samples. Of 110 MDR *P. aeruginosa*, 70 (63%) were carbapenemase and 58 (52%) were MBL producers. Antimicrobial profile of MBL producing *P. aeruginosa* reported that all isolates were resistant to β-lactams, and 89% to levofloxacin and ciprofloxacin except colistin. Of 25 (35.7%) *bla*$_{NDM}$ producing *P. aeruginosa*, 12 isolates (48%) had MIC 16µg/mL to imipenem. Of 23 (32%) *bla*$_{VIM}$ producing *P. aeruginosa*, 12 (52%) contained MIC 16µg/mL to imipenem. However, 12 (17.1%) *bla*$_{OXA-48}$ producing *P. aeruginosa*, 4 (33%) contained MIC 16µg/mL to imipenem. *In vitro* AgNPs activity inhibited and killed MBL producing isolates at 1 mg/mL and 2 mg/mL, respectively. AgNPs may be used as an alternative therapy followed by multiple clinical trials.

Keywords: *P. aeruginosa*, MBL, MIC, silver nanoparticles.

INTRODUCTION

The extensively drug-resistant *Pseudomonas aeruginosa* (XDR-PA) is an emerging, life-threatening opportunistic pathogen that has become a serious global public health issue, especially in developing countries such as Pakistan (Aguilera-Saez *et al*., 2019). As a result, it is the leading cause of clinical infections, particularly among patients admitted to intensive care units, postoperative surgical injuries, burns, trauma and pre-existing pulmonary diseases such as cystic fibrosis (Awan *et al*., 2019). They are inherently resistant to different classes of antibiotics due to the presence of an over-expressed efflux pump, low permeability of the external membrane and enzymes (Fernández and Hancock, 2012). According to the Centre for Disease Control and Prevention (CDC), over 32,600 clinical infections are reported in patients in the United States with 2,700 deaths annually (CDC, 2019). Metallo-β-lactamase (MBL) is a diversified set of enzymes that hydrolyze a wide range of β-lactam drugs, including carbapenems, with the exception of colistin or polymyxin B (Qamar *et al*., 2019). The Ambler classification divides β-lactamases into four broad classes: A, B, C and D; MBL belonging to class B that comprises IMP, VIM, GIM, SIM, OXA and NDM-1 (Qamar *et al*., 2015). Pathogens that cause *bla*$_{NDM-1}$ are responsible for many diseases that pose serious health risks and their resistance is continually increasing worldwide, particularly in Asian countries, including Pakistan, India and Bangladesh (Yong *et al*., 2009).

In 2015, the first study conducted in Pakistan revealed that the rapid spread of *bla*$_{NDM-1}$ Carbapenemases in pediatric patients resulted in the death of 4/9 children (Qamar *et al*., 2015). Another study conducted in Pakistan found that 42% of the *bla*$_{VIM}$ gene were in Gram negative MDR rods (Nahid *et al*., 2013). A recent Faisalabad study has also documented 12% of *bla*$_{VIM}$ carried as *P. aeruginosa* (Qureshi *et al*., 2018). As MDR and XDR pathogens emerge, new treatment options such as natural products, nanoparticles and new antibiotic inhibitors should be tested (Qamar *et al*., 2018).

Silver nanoparticles (AgNPs) are potential agents for controlling and inhibiting infections (Salomoni *et al*., 2017). AgNPs are a collection of atoms with a size of 1 to 100 nm, while a ‘nano' is used to display ‘one billionth' (Jeevanandam *et al*., 2018). AgNPs can attach to the bacterial external wall and invade it consequently, penetrate into the cell membrane, which leads to the expiry of cells. Free radical development when nanoparticles interact with micro-organisms and these free radicals can damage the cell membrane and create penetration that leads to rapid cell death (Wang *et al*., 2017). In addition, it was proposed that Silver ions could form through nanoparticles (Bilberg *et al*., 2012), and these Silver ions can connect with many important

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enzymes and disable them (Dakal *et al*., 2016). As a result, we assessed the potential of AgNPs an antimicrobial agent in comparison to MBL-producing *P. aeruginosa*.

MATERIALS AND METHODS

Ethical consideration

Prior to commencing the study, the ethical approval of this study was taken from the Ethics Review Board, Government College University, Faisalabad.

Sample collection

It was a cross-sectional and observational study. 200 clinical isolates of *P. aeruginosa* were collected from a different source of specimens, such as pus swabs, urine and blood from inpatients at the Tertiary Care Hospital Faisalabad from January to June 2017.

Identification of the isolates

Clinical isolates were reconfirmed by sub-culturing on blood agar, MacConkey agar, and Cetrimide agar and plates were incubated for overnight at 37°C aerobically. Isolates were identified based on cultural characteristics and colony morphology. Biochemical confirmation of *P. aeruginosa* was performed using API20NE.

Antibiotic susceptibility testing

Antibiotic susceptibility testing (AST) was conducted using the Kirby-Bauer disc diffusion assay according to the CLSI guidelines 2016. The implanted antibiotic discs (Oxoid, UK) were cefixime (5µg), cefoperazone (75µg), ceftriaxone (30µg), cefepime (30µg), amoxicillin-clavulanic acid (30µg), aztreonam (30µg), levofloxacin (15µg), ciprofloxacin (5µg), amikacin (10µg), gentamicin (10µg), piperacillin-tazobactam (110µg), doxycycline (30µg), imipenem (10µg), meropenem (10µg), tigecycline (15µg) and polymyxin B (300U). The interpretation of the inhibition zone has been performed in accordance with CLSI 2016 guidelines.

Minimum inhibitory concentration of carbapenems antibiotics

MIC (µg/mL) of imipenem and meropenem were determined using E-strip (Thermo Fisher Scientific, UK) against MDR *P. aeruginosa*.

Phenotypic detection of carbapenemase

Modified Hodge's test (MHT) was used for the detection of carbapenemase production as described previously (Qamar *et al*., 2019).

Phenotypic detection of metallo-β-lactamases

MBL production was determined by the EDTA disc synergy test as described by the CLSI 2016 guidelines (Qamar *et al*., 2019).

Molecular assays

Extraction of *P. aeruginosa* DNA was carried out using a commercially available kit (Thermo Fisher Scientific, United Kingdom). The bla_{NDM} Forward-GGTTTGGCG ATCTGGTTTTC Reverse- CGGAATGGCTCATCACGA TC 621bp. The bla_{VIM} Forward- GATGGTGTTTGGT CGCATA Reverse CGAATGCGCAGCACCAG 390bp. The bla_{OXA-48} Forward- GCGTGGTTAAGGATGAACAC Reverse- CATCAAGTTCAACCCAACCG 438bp. Multiplex PCR was performed using a 96-well PCR heat cycle to detect bla_{VIM}, bla_{OXA-48} and bla_{NDM} (Poirel *et al*., 2011). DNA amplicon was placed in 1.5% agarose gel and run at 120 volts over a 60-minute period. The amplified products were visualized by UV light trans-illuminator gel documentation system (Thermo Fisher Scientific).

Antibacterial activity of silver nanoparticles against MDR P. aeruginosa

AgNPs were commercially procured from US Research Nanomaterials, Inc., United States. Nanoparticles coated with 0.2% PVP stabilizing agent for easy dispersion, its size 20nm, actual density 10.5 g/cm3, purity 99.99%, black color, spherical and these were analyzed using the scanning electron microscope (SEM).

Preparation of the bacterial suspension

0.5MacFarland suspension of MBL producing *P. aeruginosa* was prepared in 3mL of LB broth (Oxoid, UK).

Preparation of stock solution of silver nanoparticles

The stock solution was prepared by dissolving 5 mg of AgNPs powder in 1 mL of dimethyl sulfide (DMSO) with a slight change from the previous study (Syed *et al*., 2009).

Assays for antibacterial activity

AgNPs were tested for their inhibitory action against bla_{NDM} producing *P. aeruginosa*, bla_{VIIM} producing *P. aeruginosa* and bla_{OXA-48} producing *P. aeruginosa* strains isolated from hospitalized patients using the standard test for determination of antibacterial activity.

The minimum inhibitory concentration of silver nanoparticles (mg/mL)

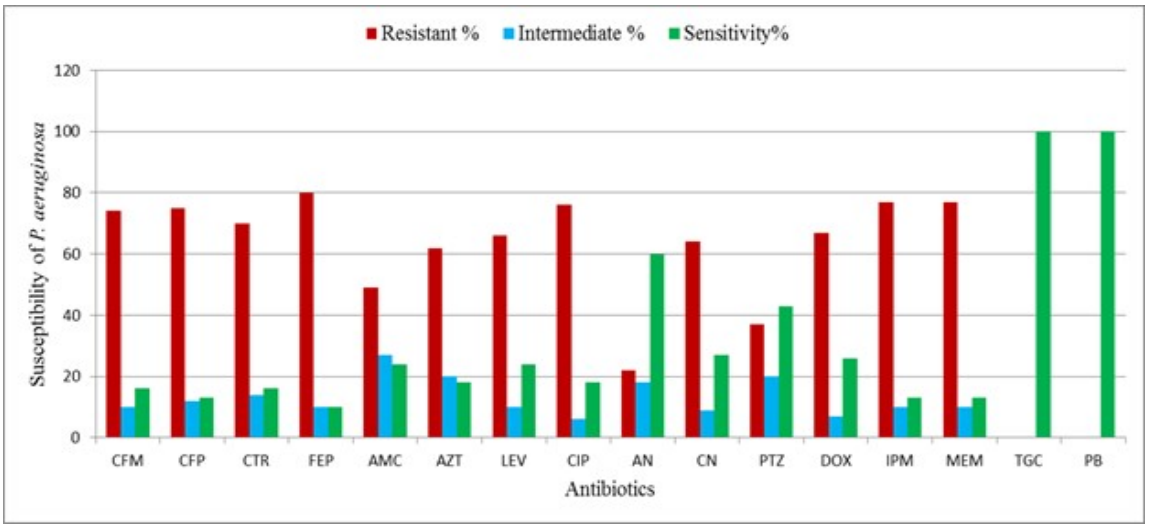
A 200µL of 0.5McFarland bacterial suspension in LB broth added in 1st to 10th well. The serial dilutions from AgNPs stock solution (5mg/mL) were added into 96 wells round bottom Microtiter plate (Thermo Fischer Scientific, UK), to achieve a final concentration of 2mg/mL to 0.0039mg/mL. Plates were incubated overnight at 37°C. The microtitration plate was visually examined to determine no growth compared each well with negative and positive controls (Syed *et al*., 2009).

Minimum bactericidal concentration

The minimum bactericide (MBC) level is defined as the first dilution with no growth on the agar plate. No visible

Table 1: Prevalence of MBL producing *P. aeruginosa* from clinical samples

Isolates	Pus	Blood	Urine	Sputum	Ear swabs	Tracheal aspirate
bla_{NDM} producing *P. aeruginosa* (n=25)	9 (12.8%)	8 (11%)	2 (2.8%)	2 (2.8%)	2 (2.8%)	2 (2.8%)
bla_{VIM} producing *P. aeruginosa* (n=23)	7 (10%)	2 (2.8%)	3 (4.2%)	3 (4.2 %)	6 (8.57%)	2 (2.8%)
bla_{OXA-48} producing *P. aeruginosa* (n=12)	3 (4.2%)	1 (1.42%)	2 (2.8%)	2 (2.8%)	4 (5.7%)	0



CFM: Cefixime; CFP: Cefoperazone; CTR: Ceftriaxone; FEP: Cefepime; AMC: Amoxicillin/clavulanate; AZT: Aztreonam; LEV: Levofloxacin; CIP: Ciprofloxacin; AN: Amikacin; CN: Gentamicin; PTZ: Piperacillin/tazobactam; DOX: Doxycycline; IMP: Imipenem; MEM: Meropenem; TGC: Tigecycline; PB: Polymyxins-B.

Fig. 1: Percentage antimicrobial susceptibility patterns of *P. aeruginosa* (n=200)

growth wells of the Microtiter plate allowed for the sampling of 10µL. It was then inoculated on nutrient agar plates (Oxoid, UK) that were aerobically incubated at 37°C for 24-hours. Plates were seen for the viability of the cells.

RESULTS

Distribution of P. aeruginosa in different clinical samples

Of 200 *P. aeruginosa*, the maximum (n=82; 41%) were isolated from pus samples, followed by 30 (15%) ear swabs, 27 (13.5%) sputum, 25 (12.5%) tracheal aspiration, 20 (10%) urine and 16 (8%) blood samples from children and adults. The ratios between men and women were, 1.5:1. The highest numbers (n=92) of *P. aeruginosa* were in the 41-60 age group, followed by 66 (61-80 years), 19 (>80 years), 18 (21-40 years) and 5 (8-20 years). The average patient age was 42.65 years, varying from 8 months to 82 years. 52 (26%) *P. aeruginosa* were obtained from burn unit, 49 (24.50%) from Intensive Care Unit (ICU), 46 (23%) from the pediatric unit, 38 (19%) from General wards, 15 (7.5) from out-door patients (OPD).

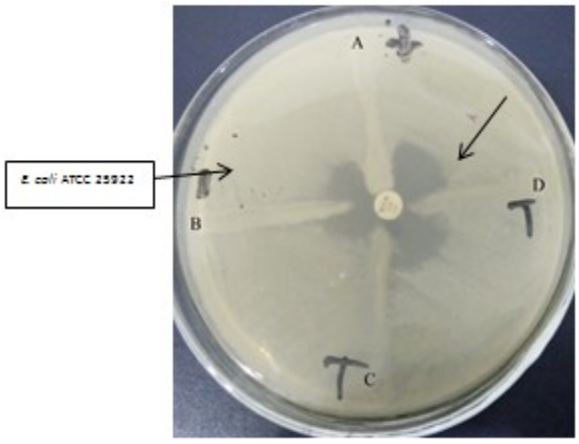


Fig. 2: shows the MHT for the detection of carbapenemase-producing *P. aeruginosa*. Black arrows show cloverleaf like an indentation. A: Positive control, B: Negative control, C and D: tested organisms.

Antibiotic susceptibility testing

A total of 110 (55%) of 200 clinical isolates were MDR *P. aeruginosa*. The antibiotic resistance pattern for *P. aeruginosa* was determined with respect to 16 different

antibiotics. 80% of *P. aeruginosa* was resistant to cefepime, 77% to carbapenem, 75% to cefoperazone, 74% to cefixime and 70% to ceftriaxone, 62% to aztreonam, whereas 50% were resistant to amoxicillin- clavulanate and 38% had lower resistance to piperacillin tazobactam. However, there was a decrease in resistance against tigecycline and colistin (fig. 1).

Phenotypic detection of carbapenemase activity

Out of 110 MDR *P. aeruginosa*, 70 (63%) were carbapenemase producers. These isolates were found in a variety of clinical samples; 25 (35.7%) pus, 17 (24.2%) ear swabs, 16 (22.8%) tracheal aspirates, 9 (12.8%) urine and 3 (4.2%) sputum samples (fig, 2).

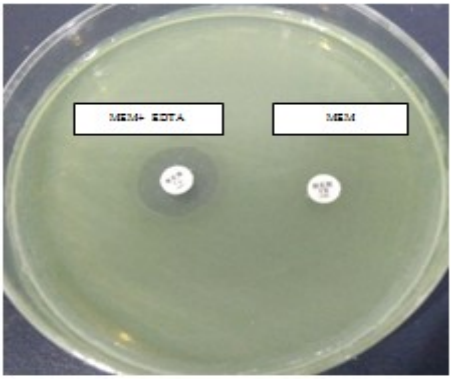
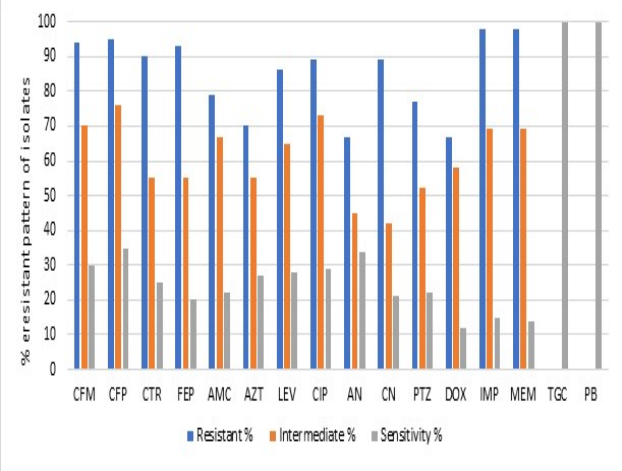


Fig. 3: shows a double-disc synergy test for the detection of MBL producing *P. aeruginosa*, MEM+EDTA disc shows a larger zone of inhibition as compared to non-EDTA MEM disc.



CFM: Cefixime; CFP: Cefoperazone; CTR: Ceftriaxone; FEP: Cefepime; AMC: Amoxicillin/clavulanate; AZT: Aztreonam; LEV: Levofloxacin; CIP: Ciprofloxacin; AN: Amikacin; CN: Gentamicin; PTZ: Piperacillin/tazobactam; DOX: Doxycycline; IMP: Imipenem; MEM: Meropenem; TGC: Tigecycline; PB: Polymyxins-B.

Fig. 4: Antibiotic susceptibility testing of metallo-β-lactamase producing P. aeruginosa (n=110).

Phenotypic detection of metallo-β-lactamase

Of the 110 MDR *P. aeruginosa*, 58 (52%) were positive for MBL production. Most of these isolates were recovered from ear swabs (25; 37.5%) followed by 15 (22%) from pus, 8 (12.5%) from tracheal aspirates, 7 (10.5%) from sputum, 6 (9%) from urine and 5 (7.57%) from blood samples (fig. 3).

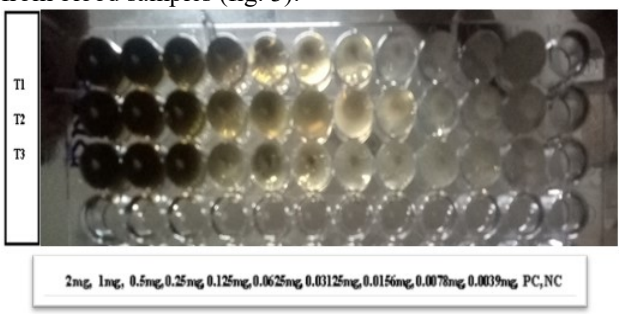


Fig. 5: Microtiter plate shows *in vitro* activity of AgNPs against T1: bla_{NDM} producing *P. aeruginosa* T2: bla_{VIM} producing *P. aeruginosa* and T3: bla_{OXA-48} producing *P. aeruginosa* PC: Positive control and NC: Negative control.

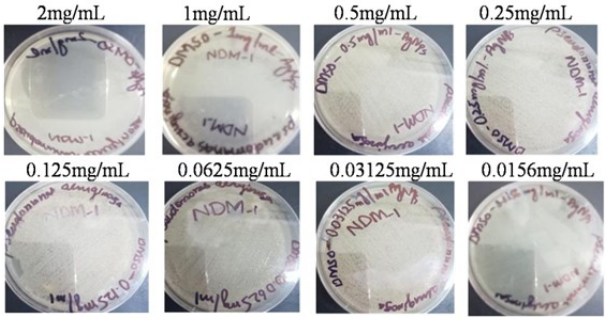


Fig. 6: *In vitro* activity of AgNPs killed bla_{NDM} producing *P. aeruginosa* at a concentration of 2mg/mL

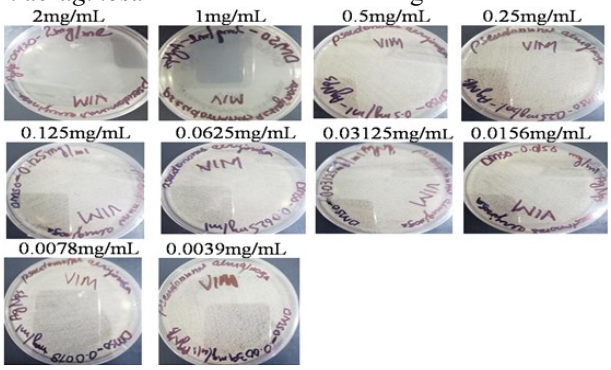


Fig. 7: *In vitro* activity of AgNPs killed bla_{VIM} producing *P. aeruginosa* at a concentration of 2mg/mL

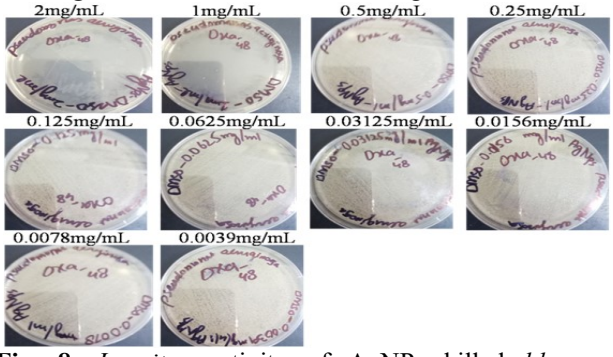


Fig. 8: *In vitro* activity of AgNPs killed bla_{OXA-48} producing *P. aeruginosa* at a concentration of 2mg/mL.

Antibiogram of MBL producing P. aeruginosa

Antimicrobial susceptibility testing of MBL producing *P. aeruginosa* revealed that 98% of the isolates were resistant to carbapenems, 95% to cefepime, 94% to ceftriaxone, 90% to cefixime. 89% to levofloxacin and ciprofloxacin while least resistance was observed against tigecycline and colistin (fig. 4).

Clinical characterization of MBL producing P. aeruginosa

Of 25 bla_{NDM} producing *P. aeruginosa*, 9 (12.8%) were isolated from pus followed by 8 (11%) from blood, and 2 (2.8%) from other clinical specimens. In addition, of 23 bla_{VIM} that *produced P. aeruginosa*, 7 (10%) were isolated from pus samples, followed by 6 (8.57%) from ear swabs and 3 (4.2%) from sputum samples. However, out of 12 bla_{OXA-48} producing *P. aeruginosa*; 4 (5.7%) were recovered from ear swabs and 3 (4.2%) from pus samples (table 1).

Minimum inhibitory concentration of MBL producing P. aeruginosa

MIC of imipenem against 25(35.7%) bla_{NDM} producing *P. aeruginosa* displayed that 12(48%) isolates were inhibited at 16µg/mL and 13(52%) at 8µg/mL. However, MIC of meropenem showed that 10(40%) isolates were inhibited at 8µg/mL and 15(60%) at16µg/mL. MIC of imipenem against 23(32%) bla_{VIM} producing *P. aeruginosa* demonstrated that 12(52%) isolates were inhibited at 16µg/mL, 7(30%) at 8µg/mL and 4(17%) at 32µg/mL. Additionally, 10 isolates (43%) were inhibited at 8µg/mL and 10(43%) at 16µg/mL and 3 (13%) at 32µg/mL against meropenem antibiotic. MIC of 12(17.1%) bla_{OXA-48} producing *P. aeruginosa* showed that 4(33%) isolates were inhibited at 16µg/mL and 6 (50%) at 8µg/mL and 2(16%) at 32µg/mL against the imipenem antibiotic. In addition, 2 isolates (16%) showed MIC at 8µg/mL and 7(58%) at 16µg/mL and 3(25%) at 32µg/mL against meropenem antibiotic.

Minimum inhibitory concentration and minimum bactericidal concentration of silver nanoparticles (mg/mL)

All the MBL producing *P. aeruginosa* were inhibited at 1 mg/mL of AgNPs concentration and killed at 2mg/mL concentration of AgNPs (fig. 5, 6, 7 and 8).

DISCUSSION

Metallo-β-lactamase production is a major antibiotic resistance mechanism among *P. aeruginosa*. The emergence of MBL producing *P. aeruginosa* in hospitals is a serious concern in the management of hospital acquired infections (Akya *et al*., 2015). In present study, 41% of *P. aeruginosa* were recovered from wound infections. These results are similar to those of the earlier study in Pakistan which reported that out of 250 children burn patients, 50 (20%) *P. aeruginosa* isolates were recovered from the swabbed burn wounds of patients with moderate to severe burned wound in different body sites (Hassuna *et al*., 2015). The prevalence of *P. aeruginosa* was higher in male patients (57.5%) than in women (42.5%) and male-female ratio was 1.5:1. Above findings are closely related to study conducted in Germany, a total of 168 patients with mean age 68.1±12.8, 113(67.3%) males and 55(32.7%) females were identified (Yayan *et al*., 2015). *P. aeruginosa* was most prevalent (46%) in patients in the age group of ≥41 to 61 years age this study shows accordance with another study in which more than 55 years old patients had the highest prevalence of *P. aeruginosa* (Ahmadi *et al*., 2016). Conceivable clarifications for the high distribution of *P. aeruginosa* in our study is due to the low levels of health care and poor sanitary conditions in the orthopedic parts of hospitals, improper prescription of operative drugs and presence of antibiotic resistance in bacterial strains (Al-Mulhim *et al*., 2014). Almost 55% of MDR *P. aeruginosa* was observed in current study which is analogous to another study in which 48 isolates (54.5%) was were documented as MDR (Saderi and Owlia, 2015). This could be due to modified target sites, activating bacterial efflux pumps, producing or reducing enzymes, loss of membrane proteins are several mechanisms involved in MDR *P. aeruginosa* (Li *et al*., 2015). *P. aeruginosa* strains were very resistant (77%) to carbapenem. These findings are consistent with an earlier study conducted in the United States that found that *P. aeruginosa* developed approximately 65% resistance to carbapenem (Labarca *et al*., 2016).

MIC values of imipenem and meropenem drugs against MDR *P. aeruginosa* isolates ranged from 8µg/mL to ≥32µg/mL. Our results are consistent with an earlier study in which the MIC of all three carbapenems was >32µg/mL while the MIC of meropenem was 16µg/mL which was greater than MIC of both imipenem (4µg/mL) and doripenem (2µg/mL) (Negi *et al*., 2017). 63% of carbapenemase producing *p. aeruginosa* among MDR-resistant isolates. These results are similar to this study in which carbapenemase producing *P. aeruginosa* were mostly hospital-acquired (71.4%) and isolated from intensive care units (66.7%) (Schäfer *et al*., 2019). In present study, 35.7% bla_{NDM} producing *P. aeruginosa* isolates were positive among *P. aeruginosa* isolates. A documented study in Lahore, Pakistan, found bla_{NDM-1} in MBL that produced *P. aeruginosa* from newborns (Qamar *et al*., 2015). Of 12 phenotypic MBL-producing *P. aeruginosa*, PCR amplification confirmed 4 (33.3%) and 3(25%) isolates harboring bla_{VIM} and bla_{IMP} gene respectively, but none carried the bla_{NDM}, bla_{SIM} or bla_{SPM} genes (Tehmasebi *et al*., 2020). A Faisalabad study reported 12% bla_{VIM} in *P. aeruginosa* (Qureshi *et al*., 2018).

In this study, 17.1% of the bla_{OXA-48} containing *P. aeruginosa* were identified as carbapenem resistant isolates of *P. aeruginosa* (CRPA). The United Kingdom

study found that 3.1% of $bla_{\text{OXA-48}}$ produced positive *P. aeruginosa* isolates (Findlay *et al*., 2017). In our clinical settings, the main risk factors associated with the spread of resistant bacteria are contaminated hands of medical personnel, various hospital surfaces, such as floors, doorknobs, sinks and intravenous catheters (Hannan *et al*., 2013). Silver possesses antibacterial properties that are too dominant mainly as nanoparticles. It has been described earlier that the antimicrobial effect of AgNPs is greater than the antimicrobial activity of silver metal alone (Kim *et al*., 2007). *In vitro* activity of MIC of AgNPs inhibited bla_{NDM} producing *P. aeruginosa*, bla_{VIM} producing *P. aeruginosa* and $bla_{\text{OXA-48}}$ producing *P. aeruginosa* at a concentration of 1mg/mL. *In vitro* activity of MBC of AgNPs killed bla_{NDM} producing *P. aeruginosa*, bla_{VIM} producing *P. aeruginosa* and $bla_{\text{OXA-48}}$ producing *P. aeruginosa* at a concentration of 2mg/mL. A study conducted in china in which MDR *P. aeruginosa* with the MIC range of 1.406-5.625 µg/mL and the MBC range of 2.813-5.625µg/mL (Liao *et al*., 2019).

As far as we know, no data are available in Pakistan on the antibacterial activity of AgNPs against bla_{NDM} producing *P. aeruginosa*, bla_{VIM} producing *P. aeruginosa* and $bla_{\text{OXA-48}}$ producing *P. aeruginosa*. AgNPs reduced the effect of QS-regulated virulence factors, pyocyanin, and elastase of *P. aeruginosa*. Moreover, these NPs have shown anti-biofilm activity marked against *P. aeruginosa*, denoting their role in reducing bacterial resistance to β-lactamases antibiotics (Rathinam *et al*., 2017).

CONCLUSION

The study concluded that MDR *P. aeruginosa* is highly prevalent in various clinical samples. These pathogens were resistant to β-lactam drugs including carbapenems. These pathogens mainly harbored bla_{NDM} and bla_{VIM} genes. To overcome this issue, the National Action Plan on AMR recommended by the Pakistani Ministry of Health needs to be actively respected. All the MBL producing *P. aeruginosa* were inhibited and killed at 1mg/mL and 2mg/mL of AgNPs, respectively. The nanoparticles may be used as a treatment alternative followed by their pharmacodynamics and pharmaceuticals.

REFERENCES

Abdul Hannan, Muhammad Usman Qamar, Muhammad Usman, Khawaja Ahmad Irfan Waheed and Rauf, K. (2013). Multidrug resistant microorganisms causing neonatal septicemia: In a tertiary care hospital Lahore, Pakistan. *Afr. J. Microbiol. Res.* **7**(19): 1896-1902.

Aguilera-Saez J, Andreu-Sola V, Larrosa Escartín N, Rodríguez Garrido V, Armadans Gil L, Sanchez García JM, Campins M, Baena Caparros J and Barret JP (2019). Extensively drug-resistant Pseudomonas Aeruginosa outbreak in a burn unit: management and solutions. *Ann. Burns Fire Disasters,* **32**(1): 47-55.

Ahmadi K, Hashemian AM, Bolvardi E and Hosseini PK (2016). Vancomycin-Resistant Pseudomonas Aeroginosa in the Cases of Trauma. *Med. Arch.,* **70**(1): 57-60.

Akya A, Salimi A, Nomanpour B and Ahmadi K (2015). Prevalence and Clonal Dissemination of Metallo-Beta-Lactamase-Producing Pseudomonas aeruginosa in Kermanshah. *Jundishapur J. Microbiol.,* **8**(7): e20980.

Al-Mulhim FA, Baragbah MA, Sadat-Ali M, Alomran AS and Azam MQ (2014). Prevalence of surgical site infection in orthopedic surgery: A 5-year analysis. *Int. Surg.,* **99**(3): 264-268.

Awan AB, Schiebel J, Bohm A, Nitschke J, Sarwar Y, Schierack P and Ali A (2019). Association of biofilm formation and cytotoxic potential with multidrug resistance in clinical isolates of Pseudomonas aeruginosa. *EXCLI J.,* **18**: 79-90.

Bilberg K, Hovgaard MB, Besenbacher F and Baatrup E (2012). *In vivo* toxicity of silver nanoparticles and silver ions in zebrafish (*Danio rerio*). *J. Toxicol.,* p.293784.

CDC (2019). *Pseudomonas aeruginosa* in Healthcare Settings. *In:* Https://www.Cdc.Gov/Hai/Organisms/Pseudomonas.Html (ed.).

Dakal TC, Kumar A, Majumdar RS and Yadav V (2016). Mechanistic basis of antimicrobial actions of silver nanoparticles. *Front. Microbiol.,* **7**: 1831-1831.

Fernández L and Hancock REW (2012). Adaptive and mutational resistance: Role of porins and efflux pumps in drug resistance. *Clin. Microbiol. Rev.,* **25**(4): 661-681.

Jeevanandam J, Barhoum A, Chan YS, Dufresne A and Danquah MK (2018). Review on nanoparticles and nanostructured materials: History, sources, toxicity and regulations. *Beilstein J. Nanotechnol.,* **9**: 1050-1074.

Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, Kim YK, Lee YS, Jeong DH and Cho MH (2007). Antimicrobial effects of Silver nanoparticles. *Nanomed-Nanotechnol.,* **3**(1): 95-101.

Labarca JA, Salles MJ, Seas C and Guzmán-Blanco M (2016). Carbapenem resistance in Pseudomonas aeruginosa and Acinetobacter baumannii in the nosocomial setting in Latin America. *Crit. Rev. Microbiol.,* **42**(2): 276-92.

Li XZ, Plesiat P and Nikaido H (2015). The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. *Clin. Microbiol. Rev.,* **28**(2): 337-418.

Liao S, Zhang Y, Pan X, Zhu F, Jiang C, Liu Q, Cheng Z, Dai G, Wu G, Wang L and Chen L (2019). Antibacterial activity and mechanism of silver nanoparticles against multidrug-resistant *Pseudomonas aeruginosa*. *Int. J. Nanomedicine,* **14**: 1469-1487.

Nahid F, Khan AA, Rehman S and Zahra R (2013). Prevalence of metallo-β-lactamase NDM-1-producing

multi-drug resistant bacteria at two Pakistani hospitals and implications for public health. *J. Infect. Public Health,* **6**(6): 487-493.

Negi A, Anand M, Singh A, Kumar A, Sahu C and Prasad KN (2017). Assessment of doripenem, meropenem, and imipenem against respiratory isolates of *Pseudomonas aeroginosa* in a tertiary care hospital of North India. *Indian J. Crit. Care Med.,* **21**(10): 703-706.

Noha A Hassuna, Amany Hosney Ibrahim Mohamed, Sahar Mohamed Aboeleuoon and Rizk HAWA (2015). High prevalence of multidrug resistant *Pseudomonas aeruginos*a recovered from infected burn wounds in children. *Arch. Clin. Microbiol.,* **6**(4): 1-7.

Poirel L, Walsh TR, Cuvillier V and Nordmann P (2011). Multiplex PCR for detection of acquired carbapenemase genes. *Diagn. Microbiol. Infect. Dis.,* **70**(1): 119-123.

Prasanth Rathinam, Kumar HSV and Viswanathan P (2017). Eugenol exhibits anti-virulence properties by competitively binding to quorum sensing receptors. *Biofouling,* **5**: 1-16.

Qamar MU, Nahid F, Walsh TR, Kamran R and Zahra R (2015). Prevalence and clinical burden of NDM-1 positive infections in pediatric and neonatal patients in Pakistan. *Pediatr. Infect. Dis. J.,* **34**(4): 452-4.

Qamar MU, Saleem S, Toleman MA, Saqalein M, Waseem M, Nisar MA, Khurshid M, Taj Z and Jahan S (2018). *In vitro* and *in vivo* activity of Manuka honey against NDM-1-producing *Klebsiella pneumoniae* ST11. *Future Microbiol.,* **13**(1): 13-26.

Qamar MU, Walsh TR, Toleman MA, Tyrrell JM, Saleem S, Aboklaish A and Jahan S (2019). Dissemination of genetically diverse NDM-1, -5, -7 producing-Gram-negative pathogens isolated from pediatric patients in Pakistan. *Future Microbiol.,* **14**(8): 691-704.

Qureshi R, Shafique M, Shahid M, Rasool MH and Muzammil S (2018). Molecular detection of blaVIM Metallo-β-lactamase producing clinically isolated *Pseudomonas aeruginosa* from tertiary care hospital, Faisalabad. *Pak. J. Pharm. Sci.,* **31**(6): 2673-2677.

Saderi H and Owlia P (2015). Detection of multidrug resistant (MDR) and extremely drug resistant (XDR) *P. Aeruginosa* isolated from patients in Tehran, Iran. *Iran. J. Pathol.,* **10**(4): 265-271.

Salomoni R, Leo P, Montemor AF, Rinaldi BG and Rodrigues M (2017). Antibacterial effect of silver nanoparticles in *Pseudomonas aeruginosa. Nanotechnol. Sci. Appl.,* **10**: 115-121.

Schafer E, Malecki M, Tellez-Castillo CJ, Pfennigwerth N, Marlinghaus L, Higgins PG, Mattner F and Wendel AF (2019). Molecular surveillance of carbapenemase-producing *Pseudomonas aeruginosa* at three medical centres in Cologne, Germany. *Antimicrob. Resist. Infect. Control,* **8**: 208.

Syed MA, Babar S, Bhatti AS and Bokhari H (2009). Antibacterial effects of silver nanoparticles on the bacterial strains isolated from catheterized urinary tract infection cases. *J. Biomed. Nanotechnol.,* **5**(2): 209-214.

Tahmasebi H, Dehbashi S, Alikhani MY, Porbaran M and Arabestani MR (2020). Prevalence and molecular typing of Metallo-β-lactamase-producing Pseudomonas aeruginosa with adhesion factors: A descriptive analysis of burn wounds isolates from Iran. *Gene Experts,* 21, 10085.

Wang L, Hu C and Shao L (2017). The antimicrobial activity of nanoparticles: Present situation and prospects for the future. *Int. J. Nanomedicine.,* **12**: 1227-1249.

Yayan J, Ghebremedhin B and Rasche K (2015). Antibiotic resistance of *Pseudomonas aeruginosa* in Pneumonia at a single university hospital center in Germany over a 10-year period. *PLoS One,* **10**(10): e0139836.

Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K and Walsh TR (2009). Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrob. Agents Chemother.,* **53**(12): 5046-5054.