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Antibacterial Efficacy of Silver Nanoparticles against Multidrug Resistant NFGNB Isolates Using Diffusion Method

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Abstract

The present study was aimed to identify, characterize and to know the antimicrobial susceptibility pattern of NFGNB from blood culture and to study the effect of AgNPs against MDR isolates. A total of 287 strains of NFGNB were isolated from 9114 blood samples from various clinical settings at Department of Microbiology, Pt. B.D.S. PGIMS, Rohtak, Haryana, India. Out of these 147(68.05%) NFGNB were Multidrug resistant strains.The most commonly isolated MDR strains included *Acinetobacter spp.* (63%) followed by *Pseudomonas spp* (18%) and *S.maltophilia* (40%). The chemically synthesized Silver nanoparticles were characterized by Fourier Transform Infrared Spectroscopy (FTIR), Transmission Electron Microscopy (TEM) and UV spectroscopy. The antibacterial activity of AgNPs was evaluated using disc diffusion assay. All tested strains were found susceptible to AgNPs. The diameter of inhibition zone was measured more than 18mm. Thus, the AgNPs can be used as an alternative to conventional antimicrobials to treat infections caused by MDR strains.

Keywords: Antibacterial, AgNPs, blood, multidrug resistance, NFGNB

Introduction

Blood stream infections (BSI) are an important cause of morbidity and mortality worldwide. Approximately 2 lakh cases of bacteremia occur annually with mortality rate ranging from 20 to 50 %. (Forbes BA *et al*., 2007). It can be potentially lethal if timely diagnosis and appropriate antimicrobial therapy is not instituted. (Diekema DJ *et al*., 2003). The problem is aggravated if the pathogenic agent belongs to the class of non-fermenters, since they are often Multidrug resistant (MDR) (Gautam V *et al*., 2015).

The studies have reported *Acinetobacter* spp., *Pseudomonas aeruginosa, Burkholderia cepacia* complex (BCC) and *Stenotrophomonas maltophilia* as common NFGNB among the positive blood culture (Samanta P *et al*., 2011). Blood culture is a vital tool for the detection of BSI and remains the gold standard for bacteremia detection (Banik A et al., 2018).

The NFGNB are aerobic, nonsporing gram negative bacilli or coccobacilli. Long duration of hospitalization and prolonged antimicrobial therapy are the predisposing factors for infection with NFGNB. Humidifiers, ventilators, dialysate fluids and catheter devices in hospital environment have provided opportunities for these organisms to establish infection (Arora *S et al*., 2012).

The increasing incidence of infections by these organisms along with the rising drug resistance necessitates their characterization up to species level and warrants a close monitoring of the antimicrobial susceptibility of these organisms.

Silver (Ag) has a strong antimicrobial potential, which has been used since the ancient times (Silver S *et a*l., 2006). Antimicrobial effects of silver can be increased by manipulating their size at nano level (Zhao *et al*., 1998). AgNPs have been applied to a wide range of products, the most important current use as antimicrobial agents to prevent infection, such as in burn and traumatic wound dressings, diabetic ulcers, coating of catheters, dental procedures, etc(Rai M *et al*., 2009). AgNPs have a very broad range of antimicrobial activity against both gram negative and gram positive bacteria (Rai MK *et al*., 2012) . The effect of AgNP was superior against Gram-negative as compared to Gram-positive organisms (Cavassin ED *et al*., 2015). Thus, AgNPs have a potential use to treat MDR NFGNB (Lara HH *et al*., 2010).

Therefore, the present study was aimed to identify, characterize and to know the antimicrobial susceptibility pattern of NFGNB from blood culture and to study the effect of AgNPs against MDR isolates.

Material and Methods

The present study was conducted in the Department of Microbiology, Pt. B.D. Sharma, PGIMS, Rohtak over a period of 6 months. The blood culture samples received in the laboratory during this period were processed by standard microbiological techniques (Collee JG et al., 2012). NFGNB isolates were identified and antimicrobial susceptibility testing was performed as per Clinical and Laboratory Standard Institute (CLSI) guidelines (CLSI, 2017). All the antimicrobial discs were procured from HiMedia Laboratories, Mumbai, India. *P. aeruginosa* ATCC 27853 was used as the control strain.

Silver nanoparticles were prepared by chemical reduction method and further characterized by UV spectroscopy, fourier transform infrared spectroscopy (FTIR) and transmission electron microscopy (TEM) (Zhang FX et al.,2016).

Preparation of AgNPs Discs

AgNPs discs were prepared by punching of holes of approximately 6mm diameter in Whatman filter paper no. 1 and were sterilized in an autoclave 121ºC at 15psi for 30 minutes. Sterile discs were placed in petri dishes approximately 5mm apart. Using a mechanical pipette, a fixed volume of 20µl silver nanoparticles was loaded on each disc one by one, taking precautions that the tip was in slight contact with the disc. The inoculum was prepared from the culture and was matched for turbidity with 0.5 McFarland standards. AgNPs antibiotic discs were placed on the inoculated MHA agar plate along with the commercially available discs for comparison of the efficacy of the AgNPs disc. (Fig no.1)

Statistical Analysis: The data collected was analyzed using SPSS software (version 20.0; SPSS Inc., Chicago, IL, USA). Frequency distribution and cross-tabulation was used to create summary tables and compare items within and across various categories.

Results and Discussion

A total of 9114 blood culture samples were received over a period of 6 months. Out of these, 287 strains were NFGNB with a prevalence rate of 3.14%. Higher incidence of NFGNB was reported for males (64.57%) as compared to females (35.42%) as shown in Table No.1. The maximum number (60.97%) of NFGNB were isolated in the age group of 0 –10 years among which 46% were from NICU as shown in Fig. No.3. This is due to under development of immune system in this age group. Similar results were shown by study done by Sharma *et al*., in which 48.22% NFGNB were isolated from neonates. On the other hand, minimum number was reported for age group of 91-100 yrs. (0.34%) as shown in Table No 1. Among the various clinical settings included in this study, most of the positive cultures were obtained from NICU with 125 cases (43.55%) followed by Pediatric wards with 94 cases (35%). The number of positive cultures from each ward are reported in Fig 3. Gupta *et al*., 2016 and Katyal *et al*., 2018 have also reported maximum blood culture positivity from NICU, which is comparable to our study.

NFGNB were found more susceptible to meropenem (84%) followed by doxycycline (71%), imipenem (67%), and least susceptible to ticarcillin (07%) as illustrated in Table No. 3. Out of 287 NFGNB isolates, the results demonstrated a total 147 (51.21%) isolates as MDR. Our findings are also in concordance with Jayapriya *et al*., 2014 who reported 75.6% of NFGNB as MDR respectively. Studies by Vijay D *et al*., and Veenu G *et al*., have reported 31% and 62% MDR respectively.

Among 147 isolates, *Acinetobacter* spp. (63.31%) was the most commonly reported MDR, followed by *Pseudomonas* spp. (18.29%). and *S. maltophilia (40%)* as shown in Fig 2. In a study done by Patwardhan *et al*., 2008, 68% of *Pseudomonas* spp. and 90% of *Acinetobacter* spp. isolated were MDR.

The MDR isolates were used further to evaluate the antibacterial activity of AgNPs. In our study, the antibacterial activity of synthesized AgNPs was determined using disc diffusion assay against all MDR strains. The zone of inhibition was more than 18mm among all MDR strains tested as shown in Fig. no.1. Another study demonstrated the antibacterial activity of AgNPs against MDR *P.aeruginosa* and *A. baumanii* isolates from clinical samples (Santos *et al*. 2016). Singh and coworkers demonstrated the antibacterial potential of AgNPs against MDR *P.aeruginosa* strains isolated in burn patients (Singh K *et al*. 2014). Kalishwaralal *et al*. 2008 demonstrated the potential anti-biofilm activity of AgNPs against *P.aeruginosa* and *Staphylococcus epidermidis.* Hwang *et al*. 2012, demonstrated the synergistic effects of AgNPs with three conventional antimicrobials (ampicillin, choramphenicol and kanamycin) against bacteria and all combinations showed effectiveness against the bacteria tested. Thus, the finding obtained in our study are in line with the existing studies indicating the distinctive effficacy of AgNPs against MDR bacterial isolates.

Conclusions

The present study showed multidrug resistance exhibited by non-fermenter isolates pose a great problem in treating these infections. The work is focused on exploring the antibacterial potential of AgNPs to combat the problem of MDR. As a result, the outcome of presented work delineates the distinctive potential of AgNPs for their use as a suitable and alternative strategy to existing antimicrobials.

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Legends Figure and Tables

Table1: Age wise distribution of NFGNB Isolate

Age group(years)	Female	Male
0-10	62	113
11-20	24	06
21-30	23	17
31-40	04	08
41-50	02	07
51-60	01	12
61-70	02	04
71-80	-	01
81-90	-	-
91-100	01	-

Table 2: Distribution of NFGNB Isolate In Clinical Setting

Department	No. of Isolate
NICU	125
Paediatric Ward	94
Medicine Ward	29
Obs/Gyne Ward	13
BPS	10
Surgery	09
RICU	05
CTVS	01
Day Care	01

Table 3: Antmicrobial Susceptibility Pattern of NFGNB Isolate

Antimicrobial Agents	Acinetobacter spp. (No.=190)	Pseudomonas spp. (No.=82)	S.maltophilia (No.=15)
Amikacin	60(31.57%)	52(63.41%)	09(60%)
Gentamicin	61(32.10%)	51(62.19%)	10(66.66%)
Ciprofloxacin	76(40%)	57(69.51%)	12(80%)
Doxycycline	136(71.57%)	-	13(86.66%)
Cotrimoxazole	84(44.21%)	-	14(93.33%)
Amoxy+Clav	33(17.36%)	-	09(60%)
Ticarcillin	-	6(07%)	-
Piperacillan+Tazo	38(20%)	45(54.87%)	12(80%)
Ceftazidime	68(35.78%)	16(19.51%)	09(60%)
Atreonam	56(29.47%)	36(43.90%)	10(66.66%)
Imipenem	121(63.68%)	59(71.95%)	13(86.66%)
Meropenem	165(86.84%)	63(76.82%)	14(93.33%)

Fig. 1: MHA plate showing the effect of SNP on MDR isolate

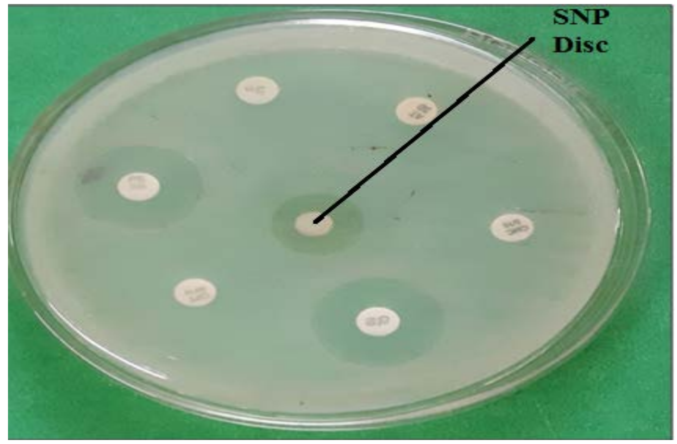

Fig. 2: Organism wise distribution of MDR NFGNB isolates

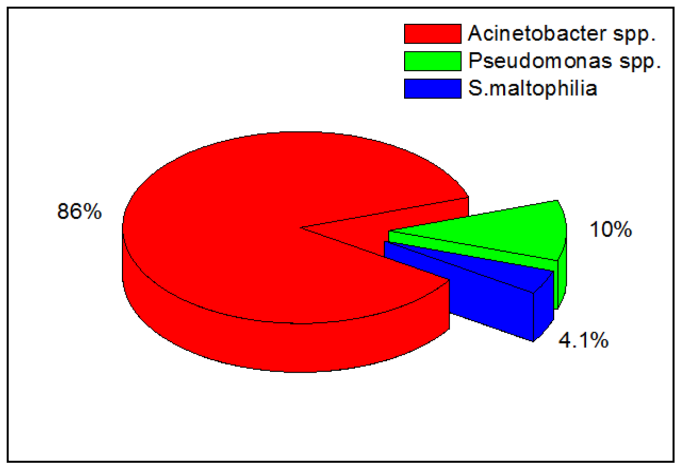

Fig.3: Distribution of NFGNB isolates in various clinical setting.