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Isolation of multidrug-resistant bacteria from the hospital environment

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ABSTRACT

Antibiotics are used to cure the illness caused by pathogenic microbes. The resistance towards such antibiotics is becoming a serious concern in the present time. The frequency of drug resistance is increasing in hospitals. This aptitude to develop resistance against antibiotics has become a serious threat to the patients that are already hospitalized, making them more prone to infections and increased complications of already existing medical conditions. It can also lead to a high mortality rate in hospitals. The present work is designed to isolate microbes from the hospital environment to check the sensitivity against various antibiotics. For the isolation, aerosol-based air samples were taken by exposing sterile Petri plate at the OPD and general ward for about 10 minutes after that the plates were taken to the lab and incubated at 37°C for 24 hours. Pure cultures were obtained by sub culturing the isolates onto fresh sterile nutrient agar plates. The clinical isolates were tested for antibiotic sensitivity test by using Dodeca G- V Plus disk (Himedia). The isolates were identified on the basis of microscopic and VITEK 2 based identification. Total of four bacteria *Kocuria kristinae*, *Sphingomonas paucimobilis*, *Pantoea spp.* and *Staphylococcus vitulinus* were isolated that showed variation in antibiotic-resistant pattern.



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INTRODUCTION

Antibiotics are used to cure the infection caused by pathogenic microbes. The resistance towards such antibiotics is becoming a serious concern in the present time. The infections caused by these resistant bacteria are more difficult to treat than caused by non-resistant bacteria, even the common infections are being difficult to treat as these antibiotics are becoming less effective (Levy, 1998). Previous data suggest that microbes such as *Staphylococcus aureus* isolated from hospitals

show resistant against Methicillin, Ampicillin, Penicillin and Vancomycin (Foxley *et al.*, 2016). Microbial quality of hospital air is one of the most important parameters to be a monitor as the patients, patient attendant, workers and others are in direct exposure. The microbial contaminated hospital air can exaggerate patient illness and increases the duration of treatment (Fekadu and Getachewu, 2015). Although, with the advancement in research and technologies it is now known that bacteria have developed mechanisms to escape the antibiotic attack. The most common mechanism of defense includes defense against antibiotic such as β -lactamases, aminoglycosides-modifying enzymes, involving modification of antibiotic surface receptor for the binding of antibiotics and development of efflux system to flip out of antibiotics (Joshi *et al.*, 2018). Immunocompromised patients are more susceptible to nosocomial infections, even exposure of other patients to such an organism can result in a worsening of the diseases (Cerceo *et al.*, 2016). The aim of present work is to check the antibiotic susceptibility test against isolated

mimcroorganisms and identification of clicinical isolates.

MATERIAL AND METHODS

Isolation of Microorganism from Hospital

For the sampling, the sterile nutrient agar plates were exposed to OPD, washroom and general ward of local hospital separately for ten minutes. After that, the plates were incubated at 37oC for 24-48 hours. After incubation, the plates were checked for the bacterial growth. The bacteria were further sub-cultured to obtain pure colonies on sterile nutrient agar plates.

Antimicrobial Susceptibility Test

The antibiotic susceptibility test was carried out over the isolated bacterial strains by using Dodeca G- V Plus disk (Himedia). The disc contained 12 antibiotics named Penicillin G (10 units), Amoxycillin(10mcg), Carbenicillin(100mcg), Methicillin (5mcg), Azithromycin(15mcg), Clindamycin(2mcg), Roxithromycin(15mcg), Lincomycin (2mcg), Vancomycin (30mcg), Rifampicin(5mcg), Teicoplanin (30mcg), Linezolid (30mcg). Bacterial inoculum was prepared as described by Lalitha, 2004 with little modification. Briefly, the bacterial inoculum of clinical isolates was prepared in normal saline with turbidity equivalent to 0.5 McFarland standards. 500µl of inoculums was spread onto the sterile Muller Hinton Agar (MHA) plates, and the antibiotic disc was placed and incubated at 37oC for 24 hours. The appearance of a clear zone was noted down (Lalitha, 2004).

Identification

The microorganism which showed antibiotic resistance against more than two antibiotics were characterised and identified on the basis of morphological characters on growth medium, Gram staining and biochemical analysis were done as described by Bergey's manual of systematic bacteriology (Holt *et al.*, 1989). For further confirmation and characterisation, the bacterial cultures were sent to IMTECH, Chandigarh for VITEK 2 based analysis.

RESULTS AND DISCUSSION

The strains isolated from the general ward, OPD and OPD washroom showed the prominent presence of four cultures. The results of Gram staining as shown in Table 1 revealed the presences of two Gram-positive bacteria (*Kocuria kristinae*, *Staphylococcus vitulinus*) and two Gram-negative bacteria (*Sphingomonas paucimobilis* and *Pantoea species*). *Kocuria kristinae* was isolated from OPD, *Sphingomonas paucimobilis* from hospital OPD washroom and two other *Pantoea species* and *Staphylococcus*

vitulinus were isolated from the general patient ward.

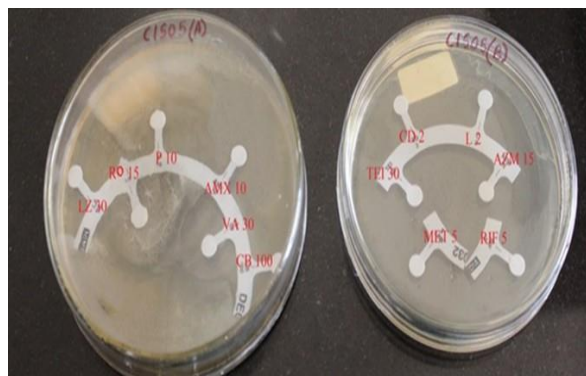


Figure 1: Represents antibiotic sensitivity test on bacterial isolate *Kocuria kristinae*

Where LZ30=Linezolid 30 mcg; RO15=Roxithromycin 15mcg; P10= Penicillin G 10 units, AMX10= Amoxycillin 10mcg; VA30= Vancomycin 30 mcg; CB100= Carbenicillin 100mcg; TEI30=Teicoplanin 30mcg; CD2=Clindamycin2mcg; L2=Lincomycin 2mcg; AZM15 =Azithromycin 15mcg; MET5=Methicillin 5mcg; RIF5=Rifampicin 5mcg; mcg= microgram.



Figure 2: Represents antibiotic sensitivity test on bacterial isolate *Staphylococcus vitulinus*

Where LZ30=Linezolid 30 mcg; RO15=Roxithromycin 15mcg; P10= Penicillin G 10 units; AMX10= Amoxycillin 10mcg; VA30= Vancomycin 30 mcg; CB100= Carbenicillin 100mcg; TEI30=Teicoplanin 30mcg; CD2=Clindamycin 2mcg; L2=Lincomycin 2mcg; AZM15 =Azithromycin 15mcg; MET5=Methicillin 5mcg; RIF5=Rifampicin 5mcg; mcg= microgram.

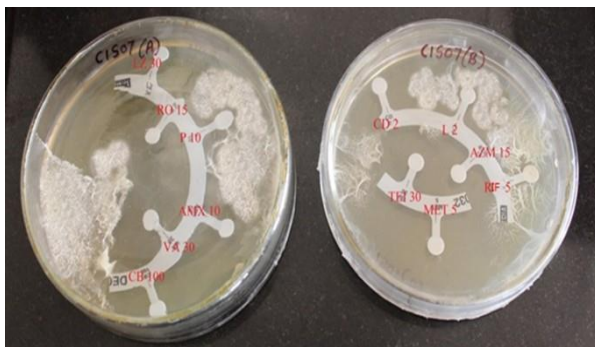


Figure 3: Represents antibiotic sensitivity test on bacterial isolate *Pantoea spp.*

Where LZ30=Linezolid 30 mcg; RO15=Roxithromycin 15mcg; P10= Penicillin G 10 units; AMX10= Amoxicillin 10mcg; VA30= Vancomycin 30 mcg; CB100=Carbenicillin 100mcg; TEI30=Teicoplanin 30mcg; CD2=Clindamycin 2mcg; L2=Lincomycin 2mcg; AZM15=Azithromycin 15mcg; MET5=Methicillin 5mcg; RIF5=Rifampicin 5mcg; mcg= microgram.



Figure 4: Represents antibiotic sensitivity test on bacterial isolate *Spingomonas paucimobilis*

Where LZ30=Linezolid 30 mcg; RO15=Roxithromycin 15mcg; P10= Penicillin G 10 units; AMX10= Amoxicillin 10mcg; VA30= Vancomycin 30 mcg; CB100= Carbenicillin 100mcg; TEI30=Teicoplanin 30mcg; CD2=Clindamycin 2mcg; L2=Lincomycin 2mcg; AZM15 =Azithromycin 15mcg; MET5=Methicillin 5mcg; RIF5=Rifampicin 5mcg; mcg= microgram.

Our results as shown in Table 2, Fig 1, 2, 3 and fig-4 indicated that these organisms had shown resistance as well as sensitivity against various antibiotics. The nosocomial infections have always been a problem for health care personals; many of the nosocomial infections have been found to be opportunistic in nature as not infecting healthy humans but can lead to a fatal condition in the immunocompromised patients. Reports are showing how these organisms have worsened the existing condition of the patient.

Kocuria kristinae formely called as *Micrococcus kristinae* is a Gram-positive bacterium. In our study, it showed the resistant against penicillin G (10 units), amoxicillin (10mcg), carbenicillin (5mcg) and roxithromycin (15mcg). *Kocuria kristinae* is considered as a pathogen to an immunocompromised patient and premature babies (Bhavsar *et al.*, 2016). Lakshmikantha *et al.*, 2015 has done case studies where he observed that 18-year-old boy when admitted to the hospital with bilateral pinpoint pupils and bilateral pulmonary crepitations, showed a decline in health conditions. The urine sample from the catheter end showed the Gram-positive bacteria, which was further confirmed as *K. kristinae* by VITEK-2 based analysis. In another study Tewari *et al.*, 2013 isolated *K. kristinae* from urine catheter of the patient that

showed resistance against penicillin, erythromycin, trimethoprim/sulfamethoxazole, ceftazidime, ceftriaxone, gentamicin, amikacin, oxacillin, ciprofloxacin, meropenem, imipenem, amoxicillin with clavulanate and vancomycin that support our study also. Similar kind of study was done by Ma *et al.*, 2005 where *K. kristinae* isolated from the bile sample of the patient who undergoes undergone Laparoscopic cholecystectomy. All the reports showed that they become functional in an immunocompromised host. In one of the study, it was found that patient with chronic hepatitis C infection suddenly developed a stroke. The reports of blood culture revealed the presence of *K. kristinae*. The antibiotic therapy could not cure the infection showing its resistance against antibiotic and the patient underwent an aortic valve replacement with mechanical prosthesis and debridement of the mitral valve. The main cause of increased complication was infection by *K. kristinae* and its resistance against antibiotics (Aleksic *et al.*, 2016), which is in corroboration with our results. Recently, Kandi *et al.*, 2016, in one of their review mentioned that *Kocuria* species belonging to family *Micrococcaceae*, which also include *staphylococcus species* was earlier not considered to be clinically significant. But, increasing the incidence of infections associated with this bacterium has made it clinically important (Kandi *et al.*, 2016). In our study *Staphylococcus vitulinus* was isolated from the general ward, which is a Gram-positive bacterium. It belongs to *Staphylococcus sciuri* group; other members include *S. sciuri* and *S. lentus*. It is coagulase negative and consists of clustered cocci. Coagulase negative *Staphylococci* (CoNS), is one of the major commensal of skin flora. Earlier, they were considered avirulent, but with the passage of time, they have been recognised as a cause of clinically significant nosocomial bloodstream infections. They are principally associated with animals, but studies revealed that they had been associated with serious infections including endocarditis, peritonitis, septic shock, urinary tract infection, endophthalmitis, pelvic inflammatory diseases and most commonly wound infection (Stepanovic *et al.*, 2003). In our study, *S. vitulinus* showed resistance against Penicillin G (10 units), Azithromycin (15 mcg) and Lincomycin (2 mcg). A similar study by Kurekci 2016, showed the resistance of microbes against various antibiotics and hence supporting our results. Stepanovic *et al.*, 2003 isolated *S. sciuri* group which includes *S. sciuri*, *S. lentus*, and *S. vitulinus* from the urine sample and observed that 50% of the isolated microbes are of hospital originated. Ahoyo *et al.*, 2013 isolated *S. vitulinus* from the hospital environment, which was resistant to PenicillinG, Oxacillin, Tetracyclin, Trimethoprim, sulfa-

Table 1: Microorganisms isolated from a different area of hospitals

Sl. No	Designation of cultures	Area of isolation (exposure to air)	Gram staining characteristics	Microscopic characteristics (100X)	Identified by VITEK 2
1	C1S05	OPD	Gram-Positive	Cocci shaped	<i>Kocuria kristinae</i>
2	C1S08	General ward	Gram-Positive	Rods	<i>Staphylococcus vitulinus</i>
3	C1S07	General ward	Gram-Negative	Cocci shaped arranged in tetrad form	<i>Pantoea species</i>
4	C1S04A	Hospital OPD Washroom	Gram-Negative	Rod shaped	<i>Sphingomonas paucimobilis</i>

Table 2: Microorganisms shown resistance and sensitivity against various antibiotics

Antibiotics used	Clinical isolates			
	<i>Kocuria kristinae</i>	<i>Staphylococcus vitulinus</i>	<i>Pantoea spp.</i>	<i>Sphingomonas paucimobilis</i>
Penicillin G P 10units	R	R	R	S
Amoxycillin AMX 10 mcg	R	S	S	R
Carbenicillin CB 100 mcg	R	S	S	S
Methicillin MET 5 mcg	S	S	S	S
Azithromycin AZM 15 mcg	S	R	S	R
Clindamycin CD 2 mcg	S	S	S	S
Roxithromycin RO 15 mcg	R	S	S	S
Lincomycin L 2 mcg	S	R	R	S
Vancomycin VA 30 mcg	S	S	S	S
Rifampicin R 5 mcg	S	S	R	R
Teicoplanin TEI 30 mcg	S	S	S	S
Linezolid LZ 30 mcg	S	S	S	S

R= Resistance; S =sensitivity

Table 3: Percentage of antibiotic resistance shown by clinical isolates against antibiotics used in the study

Isolates	Percentage of antibiotic resistance
<i>Kocuria kristinae</i>	4/12*100= 33%
<i>Staphylococcus vitulin</i>	3/12*100=25%
<i>Pantoea spp.</i>	3/12*100=25%
<i>Sphingomonas paucimobilis</i>	3/12*100=25%

metoxazole and Vancomycin. Moon *et al.*, 2012 isolated *S. vitulinus* from veterinary were SCCmec type I and IVnt, types I usually associated with methicillin-resistant *S. aureus* whereas type IV noticed in community-associated MRSA. Another strain isolated from the general ward includes the *Pantoea species* which was further they are confirmed by the VITEK 2 method. *Pantoea species* is a Gram-negative rod of *Enterobacteriaceae* family, associated with plants origin and is not an obligate infectious agent in humans. But, reports have revealed that it could be a cause of opportunistic human infection, mostly by wound infection with plant material or as a hospital acquired infection mainly in an immunocompromised host (Dutkiewicz *et al.*, 2016). In this species, the most common infectious agent includes *P. agglomerans* and is found to be responsible for septic arthritis or synovitis, Cholelithiasis, bloodstream infections, osteitis. This organism has also been reported to be a

potential candidate for powdered infant milk formulae born opportunistic pathogen (Mardaneh and Dallal, 2013). Our results revealed that the microbe was fond of being resistant against Penicillin G (10 units), Lincomycin (2mcg) and Rifampicin (5mcg). The resistance activity of the microbe has also been studied by other groups. Habsah *et al.*, 2005 studied an outbreak of death of six out of seven affected neonates caused by *Pantoea spp.* They have isolated the bacteria from the blood sample taken from the patients and found to be sensitive to antibiotics namely amikacin, cefoperazone, ceftazidime, cefuroxime, ciprofloxacin, cotrimoxazole, gentamicin, imipenem, netilmicin, piperacillin, piperacilin/tazobactam, cefepime and cefoperazone/sulbactam, but resistant to ampicillin, which is in corroboration to our results. Liberto *et al.*, 2009 reported *P. agglomerans* from the blood sample of 6 patients, who were under chemotherapy and during treatment they had of

chills and fever. They concluded that *P. agglomerans* could cause nosocomial infection in the patient with immunosuppression. All six isolates were found to be sensitive to ampicillin/sulbactam, cefepime, cefixime, cefotaxime, ceftazidime, cefuroxime, quinolones, gentamicin, imipenem, meropenem, mezlocillin, piperacillin, piperacillin-tazobactam, tetracycline, azol whereas shown resistant/intermediate to ampicillin and ceftazidime cotrimox.

The last isolated strain was identified as *Sphingomonas paucimobilis* and was confirmed by the VITEK 2 method. *S. paucimobilis* was initially known as CDC Grp III, Biotype-I and later named as *Pseudomonas paucimobilis*. Later in 1990, it was placed into its genus *Sphingomonas*. It is a yellow pigmented, non-fermenting Gram-negative rod. What differs it from other Gram-negative bacteria is the presence of *Sphingolipids* instead of lipopolysaccharide, which are capable of inducing tumour necrosis factor, IL-6 and IL-1 from the mononuclear cells (Krziwon *et al.*, 1995). It is widely distributed in the environment and has been frequently isolated from hospital equipment (Casadevall *et al.*, 1992). Reports are available showing its presence even in the distilled water, haemodialysis fluid and sterile drug solutions (Maragakis *et al.*, 2009). Initially, this organism was also thought to be non-pathogenic, but later it was found to involve in many infections. Cases have been reported for wound infection, meningitis ventilator associated pneumonia (Martino *et al.*, 2000; Mehmood *et al.*, 2018), splenic (Mohan and Railey, 2015.), urinary tract infection (Demir and Dadali, 2016) and osteomyelitis (Tai and Velayuthan, 2014). Our results (Table 1 & Table 2) revealed the presence of *S. paucimobilis* in hospital OPD washroom and was found to be resistant against Amoxicillin (10mcg), Azithromycin (15 mcg) and Rifampicin (5mcg). Similar kind of study was done by Anazi *et al.*, 2008. He observed it is resistant against penicillin, first-generation cephalosporin and sensitive to tetracycline, chloramphenicol, aminoglycosides, trimethoprim-sulfamethoxazole, and carbapenems, in our study we also found a similar pattern. Simgamsetty *et al.*, 2016 isolated Non-Fermenting Gram-Negative Bacilli and identified by using VITEK-2 compact and checked the antimicrobial sensitivity test. They have checked antibiotic sensitivity test using AST N281 card which includes Levofloxacin, Gentamicin, Cefepime, Meropenem, Imipenem, Ticarcillin/Clavulanic acid, Doripenem, Ceftazidime, Cefoperazone/Sulbactam, Amikacin, Ciprofloxacin, Minocycline, Tigecycline, Colistin, Trimethoprim/Sulfomethoxazole(Cotrimoxazole), Cefotaxime, Piperacillin/Tazobactam, Cefuroxime, Ceftriaxone, Tobramycin. A total of 186 bacterial cultures were isolated from different sources

includes tracheal aspirate, pus/wound infections, blood cultures, urine sputum and central line tip. From their study, it was found that isolated *Sphingomonas paucimobilis* is resistant to above-listed antibiotics and concluded that Vitek 2 based identification is the reliable test that reduces the time for identification of pathogen and gives quick treatment to the patient and thus reducing the mortality rate (Simgamsetty *et al.*, 2016). Table 3 represents the percentage of antibiotic resistance; the highest resistance is observed in *Kocuria kristinae* whereas as other isolates shown equal percentage.

CONCLUSION

The present study for isolation and identification of organism present in the aerosol form signifies that in the health care system now the organism which was earlier considered as nonpathogenic are now very much prevalent in a pathogenic form. Their eradication is the utmost requirement as they can result in worsening of already existing diseases in the patient. In many cases, it has also been observed that such organisms now become capable of infecting immunocompetent individuals also. Their tendency to develop resistance is a threat to mankind, and it is the need of an hour to overcome with such organisms.

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