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Isolation and Characterization of Multi drug resistant bacteria from Chidambaram GH, Cuddalore, Tamil Nadu, India

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Abstract
Antibiotic resistance is one of the biggest threats to global health, food security, and development today. Thus, the study was primarily carried out to investigate the presence of multi drug resistant bacteria in different sites like dressing materials, OT table, air, floor, labour wards, bed sheets and instruments of the Operation Theater, etc. of hospital collected from Chidambaram Government Hospital, Cuddalore, Tamil Nadu, India. Antibiotic resistant bacteria were identified by Kirby Bauer diffusion technique and are categorized as sensitive or resistant based on the zone of inhibition of antibiotics. 48 bacterial strains were selected and found *Pseudomonas* was the predominant organism in Chidambaram GH. 7 strains were found to exhibit antibiotic resistance. Among them, 2 strains were found to be multidrug resistant and were identified as *Pseudomonas aeruginosa* (Resistant to amoxicillin, streptomycin, kanamycin and Norfloxacin) and *Klebsiella pneumonia* (Resistant to amoxicillin, streptomycin, kanamycin and gentamicin). Thus, Antibiotic resistance is accelerated by the misuse and overuse of antibiotics, as well as poor infection prevention and control. Steps should be taken to reduce the impact and limit the spread of resistance.

Keywords: *Antibiotic resistance,* Pseudomonas aeruginosa, Klebsiella pneumonia

1. Introduction
Antimicrobial resistance is a major public health crisis, eroding the discovery of antimicrobials and their application to clinical medicine [9]. Antibiotics exert a selection in favor of resistant bacteria by killing or inhibiting growth of susceptible bacteria. Resistant bacteria can adapt to environmental conditions and serve as vector for the spread of antibiotic resistance. Resistance may compromise treatment, leading to increased mortality, extended hospital stays and greater healthcare costs [38]. Hence, the World Health Organization (WHO) called the year 2011, year of 'Antibiotic Resistance' [12].
Involvement of primary healthcare is particularly important as this is where almost 80% of all antibiotics used within the health service are prescribed [4, 16]. As reported, post operative infections in hospitals were mainly due to the presence of contamination of medical equipment, environmental surfaces, air and hands of health personnel [28]. Drug-resistant strains initially appeared in hospitals, where most antibiotics were being used [17].
Indiscriminate use of antibiotics for medical purposes has taken the brunt of the blame, namely, use by those physicians who prescribe antibiotics for viral infections to make their patients feel comfortable when antibiotics are known to be useless against viruses. In fact, all antibiotic use, whether medical, agricultural, and necessary or not, leads to increased resistance [15]. Diseases and disease agents that were once thought to be controlled by antibiotics are returning in new leagues resistant to these therapies [2, 17, 19]. Various microorganisms have survived for thousands of years by their ability to adapt to antimicrobial agents. They do so via spontaneous mutation or by DNA transfer. This process enables some bacteria to oppose the assault of certain antibiotics, rendering the antibiotics ineffective [13, 14].
Transmission of resistance in the hospital can occur via horizontal gene transfer between strains of bacteria that are either infectious or commensal [23]. It can also occur via transfer of bacteria between individuals, both patients and health care workers, through either direct or indirect contact [33]. Some microorganisms that are important causes of infection in humans, such as gram negative bacilli (GNB) that include *Enterobacter* spp. and *Pseudomonas aeruginosa*, are able to survive for long periods of time in the environment, thus contributing to the selection of resistant pathogens disseminated in the environment, as well as in hospitals, industry and veterinary facilities. These natural reservoirs of resistant genes may contribute to the appearance of resistant bacteria due to gene transfer mechanisms [6, 10, 35].
Practice of self medication was also a problem [1] wherein injection and antibiotic use of the hospital is to the standard of WHO limit [3]. It is believed that, these factors may serve as a selective pressure and contribute to the increase drug resistant strains in hospital environment [28].
For the increase cause of such infection in hospitals, at least two mechanisms have been documented. First, antimicrobial resistant flora may be endemic within the institution and may be transferred to the patient within the hospital setting. Second, a small population of antimicrobial-resistant bacteria that are a part of patient's endogenous flora at the time of hospitalization may emerge under the selective pressure of antibiotics and become the dominant flora [17].
The success of a hospital-centered effort to reduce antibiotic resistance depends on whether antibiotic resistance is actually evolving in hospitals. Thus, the study was primarily carried out to investigate the presence of antibiotic resistant bacteria in different sites of hospital collected from Chidambaram Government Hospital, Cuddalore, Tamil Nadu, India.

2. Materials and methods

2.1 Sampling Locations and Collection

This study was conducted in the different objects of hospital environment like dressing materials, OT table, air, floor of the OT, labour wards, bed sheets and instruments of the operation theater, suction tube, tap water, boiled water collected from Chidambaram Government Hospital, Cuddalore, Tamil Nadu, India, using sterile cotton swab. They were inoculated aseptically into sterile nutrient broth as transport medium and were transported to the laboratory within 24h for analysis.

2.2 Isolation of Bacteria

Samples were processed within 24hrs of collection. 1ml of the sample was mixed with 9 ml of sterile distilled water that representing 10^{-1} dilution and then it was serially diluted up to 10^{-10} dilution. Conventional pour plate technique was carried out for the isolation of bacterial using sterile nutrient agar with triplicates. After 48 hours of incubation at room temperature, different morphology colonies were selected and subcultured and stored at 4^0c for further use.

2.3 Grouping of Bacteria

All the bacterial isolates thus obtained were differentiated using selective and differential media such as Mannitol salt agar (MSA) for *Staphylococci*, MacConkey's agar for *Salmonella* and *Shigella* strains, Eosin methylene blue agar (EMB) for *E. coli*, *Pseudomonas* Isolation agar for *Pseudomonas aeruginosa*, Xylose lysine deoxycholate agar (XLD) for *Salmonella* and *Shigella*, *Proteus* and *Klebsiella* strains and Thiosulfate-citrate-bile salts-sucrose agar (TCBS) Agar for *Vibrio* sp.

2.4 Isolation of Antibiotic resistant bacteria

Only the conventional antibiotics regularly available for frequent use were considered for the study. The Kirby-Bauer disc diffusion technique was employed to determine the antibiotic susceptibility pattern of the isolates to the selected antibiotics such as 1.)Amoxicillin, 2.)Streptomycin, 3.)Kanamycin, 4.)Tetracycline, 5.)Chloramphenicol, 6.)Norfloxacin, 7.)Methicillin, 8.)Gentamicin and 9.)Erythromycin.

2.5 Standardization of inoculums

Pure colonies of each isolate on a 24h plate culture were randomly selected and inoculated into 2 ml of sterile peptone water broth. This was incubated at 37°C for 6hrs. 1mL of the bacteria suspension was transferred into a well dried surface of Muller-hinton agar medium and spread evenly over the entire surface of the agar plate. The antibiotic discs were then placed on the surface of the inoculated plate and incubated aerobically at 37°C for 18 to 24 h (over-night). The diameter of the zone of inhibition was measured in millimeter. The result of each antimicrobial agent tested was reported as susceptible or resistant.

2.6 Identification of Multi drug resistant bacteria:

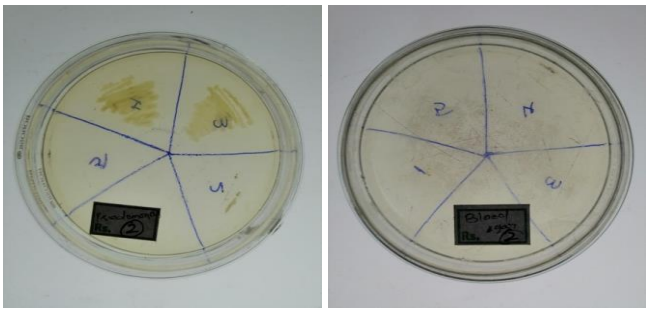
Multi drug resistant bacteria were identified by studying their cultural and morphological features from the results of Gram staining reaction and biochemical tests such as indole, methyl red test, voges proskeur, citrate, urease, oxidase, carbohydrates fermentation and gas production, catalase etc following Bergey's manual of Bacteriological classification [25].

3. Result

Isolation of bacteria from different sites (dressing materials, OT table, air, floor of the OT, labour wards, bed sheets and instruments of the operation theater, suction tube, tap water, boiled water) of hospital environments were done. From them, 48 different bacterial isolates were characterized by their growth on differential media, *Klebsiella* (1-7), *Pseudomonas* (8-22), *Enterobacter* (23-27), *E.coli* (28-33), *Vibrio* (34-37), *Staphylococcus* (38-43), *Salmonella* (44- 46) and *Shigella* (47,48)(Table 1). Among them, 41 isolates were found to be non antibiotic resistant strain and 7 strains are highly resistant to selected antibiotics. Kb-7 shows resistance to amoxicillin, streptomycin, kanamycin and gentamicin. Pd-18 shows resistance to amoxicillin, streptomycin, kanamycin and Norfloxacin. Eb-23 shows resistance to amoxicillin, erythromycin. Ec-32 shows resistance only to amoxicillin, Vb-34 shows resistance to amoxicillin and tetracycline, Vb-35 shows resistance only to amoxicillin, St-38 shows resistance to amoxicillin and methicillin. From them, Kb-7 and Pd-18 were selected based on their multidrug resistant level. They were further characterized and identified using Bergey's manual of Bacteriological classification [25].

Table 1: Organisms isolated from different objects in hospital environment

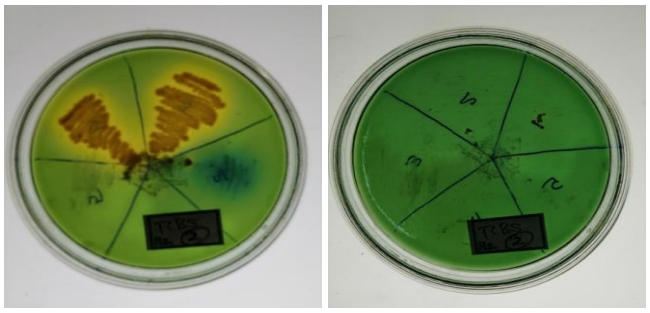
Organisms	Frequency of isolates	Sources
Klebsiella	7	OT air, table, floor
Pseudomonas	15	OT air, table, boiled water
E.coli	6	Floor, suction tube, labour ward
Vibrio	4	Dressing materials, bedsheets, tap water
Staphylococcus	6	OT air, table, floor
Enterobacter	5	Floor, suction tube, labor ward
Salmonella	3	Tap water, floor
Shigella	2	Tap water, floor



Pseudomonas Isolation agar

MacConkey agar

EMB agar



TCBS agar

Fig 1: Grouping of bacteria

Table 2: Selection of Antibiotic Resistant strain

Organism	Resistant Starin	Antibiotics
Klebsiella (1-7)	Kb-7	Amoxicillin, Streptomycin, Kanamycin, Gentamicin
Pseudomonas (8-22)	Pd-18	Amoxicillin, Streptomycin, Kanamycin, Norfloxacin
Enterobacter (23-27)	Eb-23	Amoxicillin, Erythromycin
E.coli (28-33)	Ec-32	Amoxicillin
Vibrio (34-37)	Vb-34 Vb-35	Amoxicillin, Tetracycline Amoxicillin
Staphylococcus (38-43)	St-38	Amoxicillin, Methicillin
Salmonella (44- 46)	-	-
Shigella (47,48)	-	-

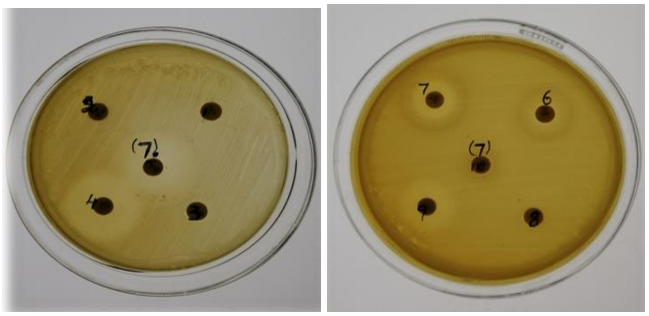


Fig 2: Plate showing *Klebsiella pneumonia* resistant to Amoxicillin, Streptomycin, Kanamycin and Gentamicin

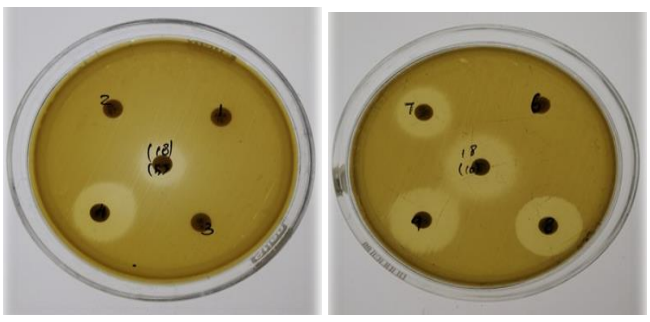


Fig 3: Plate showing *Pseudomonas aeruginosa* resistant to Amoxicillin, Streptomycin, Kanamycin and Norfloxacin

Table 3: Identification of Multi drug resistant bacterial strains

Biochemical Test	*Klebseilla pneumonia* Kb- 7	*Pseudomonas aeruginosa* Pd-18
Gram staining	Gram negative	Gram negative
Motility	Non-motile	Motile
Indole test	-	-
Methyl red	-	-
Voges proskeur test	+	-
Citrate utilization test	+	+
Urease test	+	-
Oxidasetest	+	+
Carbohydrate fermentation	-	-
Gas production	+/-	-
Catalase test	+	+

4. Discussion

Antibiotic resistance is now being recognized as a worldwide crisis in modern medicine. Infections caused by these strains are often associated with high mortality rates, prolonged hospitalization and costs [21]. In the context of antibiotic resistance, a 'successful' bacterial strain should be an extremely effective vehicle for the dissemination of antibiotic resistance traits [32].

In the present study, an attempt was made to explore the pattern of antibiotic resistant organisms responsible for hospital acquired infection in Chidambaram Government Hospital, Cuddalore, Tamil Nadu, India.

Isolation of different organisms from different objects of the hospital environment was done. From them, *Pseudomonas sp* was the major isolate from the OT air, table, boiled water and second major was *Klebsiella sp* from the OT air, table, floor. The isolated organism from the possible source showed a wide range of resistance to antibiotics used in the hospital.

The high rate of antibiotic resistance of isolated organism in our study might be due to wide spread use of antibiotics in hospitals [29]. WHO agreed on grouping the pathogens according to the species and the type of resistance and then stratifying the results in three priority tiers: critical, high and medium. Among them, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* are placed under the first priority critical group [22].

β-lactam antibiotics (beta-lactam antibiotics) are a class of antibiotic that includes penicillin derivatives, cephalosporin, monobactam, and carbapenem. Amoxicillin is a penicillin antibiotic that fights bacteria in the body. Amoxicillin in combination used to treat many different infections caused by bacteria, such as sinusitis, pneumonia, ear infections, bronchitis, urinary tract infections, and infections of the skin. It acts by inhibiting the synthesis of the peptidoglycan layer of bacterial cellwall.

The aminoglycoside class of antibiotics consists of many different agents. They are kanamycin, gentamicin, tobramycin, amikacin, plazomicin, streptomycin, neomycin, and paromomycin, which are approved by the US Food and Drug Administration (FDA) and are available for clinical use. The most common clinical application (either alone or as part of combination therapy) of the aminoglycosides is for the treatment of serious infections caused by aerobic gram-negative bacilli. While less common, aminoglycosides (in combination with other agents) have also been used for the treatment of select gram-positive infections. The

aminoglycosides primarily act by binding to the aminoacyl site of 16S ribosomal RNA within the 30S ribosomal subunit, leading to misreading of the genetic code and inhibition of translocation. The initial steps required for peptide synthesis, such as binding of mRNA and the association of the 50S ribosomal subunit, are uninterrupted, but elongation fails to occur due to disruption of the mechanisms for ensuring translational accuracy.

Quinolone group of drugs include nalidixic acid, cinoxacin, norfloxacin, ofloxacin, ciprofloxacin, levofloxacin, etc. They are effective against both Gram-negative and Gram-positive bacteria. Quinolones exert their antibacterial effect by preventing bacterial DNA from unwinding and duplicating. Specifically they inhibit the ligase activity of the type II topoisomerases, gyrase and topoisomerase IV which cut DNA in order to introduce supercoiling and with their ligase activity disrupted release DNA with single and double strand breaks which lead to cell death. For many gram-negative bacteria, DNA gyrase is the target, whereas topoisomerase IV is the target for many gram-positive bacteria.

Pseudomonas aeruginosa is a major cause of nosocomial infection is responsible for 10% of all hospital-acquired infections [5, 31]. It is the second most common causative agent of nosocomial infections [18]. But, in the present study, *Pseudomonas aeruginosa* is the first main causative agent of nosocomial infection in Chidambara GH, Cuddalore, Tamil Nadu, India. It is problematic because of its natural resistance to many drug families and its ability to develop resistance to further agents [37]. Reported rates of resistance vary greatly with period, location and type of isolates. However, almost all the single-site time course studies indicate a steady increase in resistance to antipseudomonal drugs. Worrying rates of MDR *P. aeruginosa* have been reported from the Kingdom, including strains with carbapenemases as well as ESBLs [38].

Nowadays, the prevalence of *Pseudomonas aeruginosa* and the new resistant strains continue in both community-acquired pathogens and hospital originated infections. Previous studies suggest that the selective pressure from the use of antimicrobial agents is a major determinant for the emergence of resistant strains [30]. One of the significant resistant groups detected against aminoglycosides was *Pseudomanas aeruginosa*. In various studies, it was reported that increased resistance rates have been detected against to carbapenems, quinolons and third-generation cephalosporins for *Pseudomanas aeruginosa* worldwide [24, 34].

A resistance rate of 31.5% by *Pseudomonas aeruginosa* was found for norfloxacin in one study [38]. Taking MDR as resistance to more than 3 classes of antibiotics, it was found the trait in 1–2% of inpatient isolates from the Dhahran region but in none of the outpatient strains [7]. A study from Riyadh found multiresistance in 3 and 2% of isolates collected in 2004 and 2005, respectively [11]. More recently, multidrug resistance in 21% of *P. aeruginosa* was found from an ICU in Riyadh in 2009 [36].

Klebsiella pneumonia is a natural inhabitant of the gastrointestinal tract microbiome of healthy human and animals. It is a common opportunistic hospital-associated pathogen, accounting for about one third of all Gram-negative infections overall. Data retrieved from the European Antimicrobial Resistance Surveillance Network, shows non-susceptible rates for *K. pneumoniae* and *Escherichia coli*, against the four major antibiotic classes: the third-generation cephalosporins, aminoglycosides, fluoroquinolones and carbapenems [32].

Present study reports that the *Klebsiella pneumonia* is resistant to amoxicillin, streptomycin, kanamycin and gentamycin. Older studies suggest that up to 63 and 33%, of *Klebsiella* are now resistant to amikacin, gentamicin, and tobramycin respectively [8, 36]. Similar to the present finding, *Klebsiella pneumonia* was found to resistant to ampilcillin, tetracycline, streptomycin, gentamicin and kanamycin in previous report [26]. Resistance of gram-negative aerobic bacteria to aminoglycoside antibiotics differs according to region and country [37]. Clinical isolates of *K.pneumoniae* are generally resistant to a wider range of antibiotics, and virtually always naturally resistance to ampicillin and amoxicillin [27]. In most Indian studies *Klebsiella sp.*, occupies second place. However in the present study also they were to be the second common pathogen in causing hospital acquired infection. It can spread rapidly between patients in healthcare settings and is a frequent cause of hospital outbreaks [20].

Effective antibiotic stewardship in both clinical and community settings is important to reduce selective pressure, and to slow or prevent the proliferation of resistant strains. Further actions to ensure the strict implementation of the already existing legislation to prohibit the sale of antibiotics without prescription in the Kingdom are needed. Thirdly, strict infection control measures should also help to slow the spread of resistant Gram negative pathogens in hospitals [38].

5. Conclusion

From the current study, *Pseudomonas aeruginosa* was found to be the major causative agent of hospital acquired infection in Chidambaram GH, Cuddalore, Tamil Nadu, India. *Klebsiella pneumonia* was found to be the second top organism responsible for hospital acquired infection. Thus, a strict infection control measure has to be developed to control the spread of pathogens in hospitals.

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