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Comparative Study of Chemical and Herbal Disinfectants on Multidrug Resistant *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa acts as an opportunistic pathogen because of its ability to survive on inert materials, its minimal nutritional requirement and its resistance to antimicrobial agents and antiseptics. The aim of the present study was to study the comparative investigation of chemical and herbal disinfectants on multidrug resistant *Pseudomonas aeruginosa*. All the *Pseudomonas aeruginosa* were tested against 10 different antibiotics as well as various types of disinfectants. All *Pseudomonas aeruginosa* (100%) strains were resistant to Cefuroxime, 80% strains were resistant to Amoxyclave, 50% each to Cephadroxil and Trimethoprim, 40% each to Azithromycin, Cefixime, Gatifloxacin and Levofloxacin, 30% each were resistant to Doxycycline hydrochloride and Sparfloxacin. It was found that all the strains (100%) were resistant to Hydrogen Peroxide and Potassium permanganate, 70% to Alcohol and 50% to Formaldehyde. Except Pine oil (70% resistant), all the strains (100%) were resistant to Cow urine, Lavender oil and Peppermint oil. Out of four herbal extracts analysed (Chitrak, Daruhaldi, Hirda and Neem), the only extract to which 100% sensitivity was observed was Hirda.

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Introduction

Pseudomonas aeruginosa is a nosocomial pathogen responsible for 10-15% of nosocomial infections and 65% of mortality in hospitals all over the world (Strateva *et al.*, 2007). Although it causes diseases in healthy individuals, it is a major threat to hospitalized and immunocompromised patients. The high mortality is associated with its infections and its resistance to antibiotics (Meenakumari *et al.*, 2011). *Pseudomonas aeruginosa* acts as an opportunistic pathogen because of its ability to survive on inert materials, its minimal nutritional requirement and its resistance to antimicrobial agents and antiseptics (Ndip *et al.*, 2005; Kamel *et al.*, 2011). The contaminated respiratory care equipment, irrigating solutions, catheters, infusions, dilute antiseptics, cleaning liquids, and even soaps have been reported as vehicles of transmission of *Pseudomonas aeruginosa*.

The majority of *P. aeruginosa* strains are resistant to most of antibacterial agents and disinfectants it is considered as one of the major problems (Kamel *et al.*, 2011). These resistant strains establish themselves in the hospital environment in areas like sinks, taps, railing, mattress, toilets, and thereby spread from one patient to another (Haghi *et al.*, 2010). To combat this bacterial pathogen the use of cow urine, some essential oils as well as some herbal extracts can be used. Cow urine possesses antimicrobial properties against *Pseudomonas*

aeruginosa (Ahuja *et al.,* 2012). Apart from this antimicrobial activity of some essential oils such as Peppermint oil (*Mentha piperita*), Pine oil (*Pinus sylvestris*) and Lavender oil (*Lavandula angustifolia*) has been reported. Plant essential oils have been used for hundreds of years as natural medicines to combat pathogens, including bacteria, fungi and viruses (Hammer *et al.,* 1999). Various medicinal plants have been used for years in daily life to treat diseases all over the world. Chitrak (*Plumbago zeylanica*) (Kaur *et al.,* 2017), Daruhaldi (*Berberis aristata*) (Moholkar and Worlikar, 2013), Hirda (*Terminalia chebula*) (Monisha *et al.,* 2013) and Neem (*Azadirachta indica*) (Mistry *et al.,* 2014) are reported to have antimicrobial activity. Therefore the aim of the present project was to study the comparative investigation of chemical and herbal disinfectants on multidrug resistant *Pseudomonas aeruginosa*.

Materials and Methods

Pseudomonas aeruginosa Isolates

A total of 10 *Pseudomonas aeruginosa* isolates were obtained from pathology laboratory in Nagpur. All the isolates were further identified on the basis of morphological, cultural and biochemical characteristics (Collee and Marr, 1996) and were further used for the study.

Chemical and Herbal Disinfectants Used in the Study

In the present study 4 types of chemical disinfectants (Alcohol, Formaldehyde, Hydrogen peroxide and Potassium permanganate), 4 types of medicinal disinfectants (Cow urine, Lavender oil, Pine oil and Peppermint oil) as well as 4 types of herbal disinfectants (Chitrak, Daruhaldi, Hirda and Neem) were analysed against all *Pseudomonas aeruginosa* isolates.

Antibiotic sensitivity test

The antibiotic susceptibility pattern of all the collected *Pseudomonas aeruginosa* was tested by 10 antibiotic discs obtained from Hi-media Laboratories Pvt. Ltd. Mumbai (Table 1) and Agar Well Diffusion Method (Bauer *et al.,* 1996).

Disc Diffusion Method

In brief, *Pseudomonas aeruginosa* isolates were grown overnight on nutrient agar at 37^0C, and the colonies were suspended in sterile saline water equivalent to a 0.5 McFarland standard (1.5×108 CFU/ml). The suspension (100 µL) was spread over the Mueller-Hinton agar. Then, the antibiotic disc was transferred aseptically on to the surface of the inoculated Mueller Hinton agar plates, and the plates were incubated at 37^0C for 18 hours. The diameter of the zone of inhibition produced by each antibiotic disc was measured and recorded, and the isolates were classified as "resistant" or "sensitive" based on the standard interpretative chart according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2007).

Agar Well Diffusion Method

Pseudomonas aeruginosa isolates were grown overnight on nutrient agar at 37^0C, and the colonies were suspended in sterile saline water equivalent to a 0.5 McFarland standard (1.5×108 CFU/ml). The suspension (100 µL) was spread over the Mueller-Hinton agar. The wells of 6 mm diameter were cut into the agar medium with a sterilized cork borer. Then 20µl of the chemical and herbal disinfectants were added separately into the separate wells. The plates were incubated at 37^0C for 18 hours. The diameter of the zone of inhibition around each well was measured and recorded (Bauer *et al.,* 1996).

Results and Discussion

In the present study, all the *Pseudomonas aeruginosa* were tested against 10 different antibiotics. The 100% strains were resistant to Cefuroxime, 80% strains were resistant to Amoxyclave, 50% each to Cephadroxil and Trimethoprim, 40% each to Azithromycin, Cefixime, Gatifloxacin and Levofloxacin, 30% were resistant to Doxycycline hydrochloride and Sparfloxacin (Table 2). The results indicated that *P. aeruginosa* was less susceptible to commonly used antimicrobial drugs which is an indication of circulating high levels of drug resistant strains. Resistance of *P. aeruginosa* to commonly used antimicrobial agents is becoming an increasing clinical problem and a recognized public health threat (Meenakumari *et al.,* 2011).

The chemical disinfectants such as Alcohol, Hydrogen Peroxide, Potassium permanganate and Formaldehyde were also analysed against *Pseudomonas aeruginosa.* It was found that 100% isolates were resistant to Hydrogen peroxide and Potassium permanganate, 70% isolates were resistant to Alcohol and 50% to Formaldehyde (Table 3).

Table.1 Antibiotics Discs Used in the Study

Antibiotics	Concentration	Antibiotics	Concentration
Amoxyclav	30 mcg	Doxycycline hydrochloride	30mcg
Azithromycin	15mcg	Gatifloxacin	5mcg
Cefixime	5mcg	Levofloxacin	5mcg
Cefuroxime	30mcg	Sparfloxacin	5mcg
Cephadroxil	30mcg	Trimethoprim	5mcg

Table.2 Antibiotic Resistance Profile of *Pseudomonas aeruginosa*

Antibiotics	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Amoxyclav	R	R	R	R	R	R	11mm	R	R	18mm
Azithromycin	20mm	22mm	R	R	R	16mm	22mm	16mm	22mm	R
Cefixime	22mm	R	R	R	R	12mm	18mm	14mm	14mm	12mm
Cefuroxime	R	R	R	R	R	R	R	R	R	R
Cephadroxil	R	R	R	R	R	18mm	12mm	16mm	16mm	12mm
Doxycycline	18mm	12mm	R	R	R	12mm	22mm	12mm	12mm	14mm
Glatifloxacin	24mm	20mm	R	R	R	22mm	34mm	26mm	26mm	R
Levofloxacin	26mm	22mm	R	R	R	26mm	12mm	28mm	28mm	R
Sparfloxacin	26mm	22mm	R	R	R	22mm	12mm	24mm	24mm	12mm
Trimethoprim	18mm	R	R	R	R	12mm	R	12mm	12mm	22mm

Where, P= *Pseudomonas* spp., R= Resistant

Table.3 Chemical Disinfectant Sensitivity of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa	**Alcohol**	**Formaldehyde**	**Hydrogen Peroxide**	**Potassium Permanganate**
P1	12mm	32mm	R	R
P2	R	30mm	R	R
P3	10mm	R	R	R
P4	R	30mm	R	R
P5	10mm	R	R	R
P6	R	R	R	R
P7	R	R	R	R
P8	R	36mm	R	R
P9	R	R	R	R
P10	R	10mm	R	R

Table.4 Medicinal Disinfectant Sensitivity of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa	**Cow Urine**	**Lavender oil**	**Pine oil**	**Peppermint oil**
P1	R	R	R	R
P2	R	R	14mm	R
P3	R	R	R	R
P4	R	R	R	R
P5	R	R	12mm	R
P6	R	R	R	R
P7	R	R	12mm	R
P8	R	R	R	R
P9	R	R	R	R
P10	R	R	R	R

Table.5 Herbal Disinfectant Sensitivity of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa	Chitrak	Daruhaldi	Hirda	Neem
P1	R	R	28mm	R
P2	R	R	24mm	R
P3	R	R	24mm	R
P4	R	R	24mm	R
P5	R	R	24mm	R
P6	R	R	24mm	R
P7	R	R	18mm	R
P8	R	R	22mm	R
P9	R	R	18mm	R
P10	R	R	18mm	R

Hydrogen peroxide (H2O2) is a widely used biocide for disinfection and antisepsis. Alcohols exhibit rapid broad-spectrum antimicrobial activity against bacteria. It is widely used for hard-surface disinfection. As indicated by McDonnell and Russell, reduced susceptibility of *P. aeruginosa* to any disinfectants can be associated with the ability of the bacterium to form biofilms. Growth within biofilms gives rise to extensive genetic diversity that, in turn, enhances the potential for resistance against disinfectants, which can be attributed to reduced access of antiseptic or disinfectant to underlying cell, modulation of the microenvironment and genetic exchange between cells in a biofilm, which enhances tolerance to antiseptics and disinfectant (McDonnell and Russell, 1999).

Cow urine, Lavender oil, Pine oil and Peppermint oil were also taken for the study which revealed that 100% isolates were resistant to Cow urine, Lavender oil and Peppermint oil. However, 70% were resistant to Pine oil (Table 4).

Some herbal extracts such as Daruhaldi, Neem, Hirada and Chitrak were analysed for its effect on *Pseudomonas aeruginosa*. The 100% isolates were resistant to Chitrak, Daruhaldi and Neem each. The only extract to which 100% sensitivity was observed was Hirda (Table 5).

In the present study, it was found that the *Pseudomonas aeruginosa* strains were mostly resistant to the tested disinfectants. The reduced susceptibility of *P. aeruginosa* to any disinfectants can be associated with the ability of the bacterium to form biofilms. Growth within biofilms gives rise to extensive genetic diversity that, in turn, enhances the potential for resistance against disinfectants, which can be attributed to reduced access of antiseptic or disinfectant to underlying cell, which enhances tolerance to antiseptics and disinfectant (McDonnell and Russell, 1999).

The present results indicated that *P. aeruginosa* was less susceptible to commonly prescribed antimicrobial drugs which are an indication of circulating high levels of drug resistance strains. The reduced susceptibility probably occurs as a result of indiscriminate and constant use and misuse of disinfectants. It was found to be more or less resistant to most of the antibiotics, chemical disinfectants and essential oils. The only extract to which 100% sensitivity of *Pseudomonas aeruginosa* was observed was Hirda.

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