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Study of Antibiotic Susceptibility Profile of Multidrug Resistant Microorganisms *Shigella flexneri, Pseudomonas aeruginosa* and *Staphylococcus aureus*

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ABSTRACT

In the present study isolation of pathogenic organisms were carried out. Among which multidrug resistant organisms such as shigellosis caused *Shigella flexneri*, nosocomial infection caused by *Pseudomonas aeruginosa* and *Staphylococcus aureus* were studied. Morphological and biochemical characterization was performed. Eleven antibiotics were used to inhibit isolates for sensitivity. Pathogen were tested against selected antibiotics. Shigella caused resistivity to penicillin and other four drugs of which *Pseudomonas aeruginosa* effected resistivity to ten other drugs. Minimum inhibitory concentration of *Pseudomonas aeruginosa* against different concentration of amikacin was studied. Similarly, *Staphylococcus aureus* also revealed resistance to higher concentration of amikacin exhibiting four µg/ml. MAR index analysis patterns of *Shigella flexneri* and *Staphylococcus aureus* were studied. Minimum inhibitory concentration of amikacin against *Staphylococcus aureus* revealed resistant to antibiotics. To study the plasmid profile of the multidrug resistant isolates.

INTRODUCTION

Diarrhea is a significant health problem worldwide, especially in the developing world in which sanitation facilities were lacking (Okeke, 2000). Globally diarrhoeal disease indicates for almost one fifth of all deaths of children below five years of age, with an estimate of 2.2 million deaths yearly annually. Infectious diseases kill about 11 million children each year and 99 % of these death occur in the developing countries. (Black, *et al*., 2003).

Shigella flexneri have obtained multiple antimicrobial resistance. The challenge for clinical management is distinguishing that which drug preserve their activity and clinical efficacy. The center for disease control and prevention (CDC) have suggested that sensitivity testing was accomplished to instruct selection of proper antimicrobial therapy for *Shigellosis. Pseudomonas aeruginosa* was known for its ability to resist killing by a variety of antibiotics. *Pseudomonas aeruginosa* was primarily a nosocomial pathogen. In the annual surveillance of nosocomial infection by the Centers for Disease Control (CDS), and prevention from 1990 to 1996, it is the second most common etiology of nosocomial Pneumonia; 3rd for urinary tract infection and 4th for surgical site infection (Cordero, *et al.*, 2010).

The prevalence of resistance in *Staphylococcus aureus* also is increasing globally. *Staphylococcal* resistance to penicillin is mediated by penicillin's production; an enzyme which breaks down the β-lactam ring of the penicillin molecule (Bassetti *et al*., 2009). The identification of possible associations between biofilm production and pathogenesis as well as antibiotic susceptibility profiles of infectious *Staphylococcus aureus* could provide better control measures particularly among immune compromised individuals.

Hence, the present investigation was carried out to isolate and identify the *Shigella flexneri, Pseudomonas aeruginosa* and *Staphylococcus aureus* obtained from clinical samples obtained from Government Hospital, Madurai. In addition, the sensitivity/resistance pattern of the isolates against eleven antibiotics were also performed. The aim was to collect clinical samples such as urine and stool from diseased patients and to isolate microorganisms from clinical samples and to characterize the microorganisms by morphological and biochemical characterization test. Also, present work was performed to determine the haemolytic activity of the isolated microorganisms in blood agar, study the resistance pattern of the isolates using different antibiotics and to determine its MAR index, determine the Minimum Inhibitory

Concentration (MIC) of the multidrug resistant pathogens using antibiotics and study about the plasmid profile of the multidrug resistant isolates.

MATERIALS AND METHODS

Sample collection

In the present investigation, different clinical samples such as urine and Diarrhoeal, samples were collected from Government hospital, Madurai. Diarrhoeal samples were collected from patients aseptically with the help of sterile wide mouthed screw capped plastic containers.

Isolation of Microorganisms

The different clinical specimens received from the government hospital were cultured and plated on different agar medium such as Nutrient agar, *Salmonella*, *Shigella*, Cetrimide agar, Macconkey agar and Mannitol agar. The plates were cultured and incubated at 37^0C for 24hours, after incubation, the colony characteristics were studied.

Identification of the Bacteria

Morphological and Biochemical Characterization

Morphological characteristics such as abundance of growth, pigmentation, optical characteristics, size and shape of the colony were studied on different agar medium. The efficient multidrug resistant microorganisms were biochemical characteristics by Indole, Methyl red, Voges Proskauer test, Citrate utilization test, Triple sugar Iron test, Starch Hydrolysis, Gelatin Hydrolysis, and Nitrate Reduction test.

Hemolytic Assay

The isolated strains were screened for their hemolytic activity on blood agar plates containing 5% (V/V) sheep blood and the plates were incubated at 37^0C for 24 hours. Haemolytic activity was detected as the occurrence of defined clear zone around a colony.

Determination of Multiple Antibiotic Resistances (MAR)

The resistance pattern of the isolates was determined by the disc diffusion method of Bauer *et al.,* (1996) using Muller Hinton Agar (MHA) medium. The organisms were inoculated with test samples using cotton swab. The antibiotics such as Penicillin (3oμg),

Gatifloxacin(5µg), Cefotaxime(30µg), Levofloxacin(5µg), Penicillin(2µg), Co-Trimazole(25µg) Streptomycin(10µg), Nalidixic acid (30µg), Tobramycin (10µg), Ampicillin(10µg) and Vancomycin(30µg) were placed on the inoculated agar immediately, and the plates were incubated at 37 °C for 24 hours. After incubation, the size of inhibitory zones were measured.

Determination of Minimum Inhibitory Concentration

Muller Hinton Agar medium was prepared and sterilized pour the medium on the sterile petri plates and allow it to solidify. The test organisms were swabbed on to the surface of agar plates using a sterile cotton swab. Different concentration of Amikacin (128µg/ml, 64µg/ml, 32µg/ml, 16µg/ml, 8µg/ml, 4µg/ml, 2µg/ml, 1µg/ml, 0.5µg/ml, 0.25µg/ml) discs was impregnated on to the swabbed surface. Incubate the plates at 37^0C for 24 hours. After Incubation were measured and compared with standard interpretative chat.

Plasmid DNA Isolation by Alkaline Lysis Method

Isolation of plasmid DNA was performed by using alkaline lysis method. Accordingly, three complex solutions were prepared. The isolation of plasmid DNA obtained was run on 0.8% agarose gel electrophoresis using ethidium bromide.

RESULTS

The present investigation was carried out to isolate and identify the *Shigella flexneri, Pseudomonas aeruginosa* and *Staphylococcus aureus* from clinical samples obtained from Government hospital, Madurai. The isolates were identified for their sensitivity/ Resistant pattern against eleven antibiotics were recorded.

Isolation of microorganisms from clinical samples

The different clinical specimens (Urine, Diarrhoeal) received from the Government hospital, Madurai were cultured and were plated on different agar media such as Nutrient agar, Salmonella Shigella (SS) agar, Cetrimide agar, Mannitol salt agar, and Macconkey agar in order to isolate *Shigella flexneri, Pseudomonas aeruginosa* and *Staphylococcus aureus.*

Identification of *Shigella flexneri and Pseudomonas aeruginosa*

Shigella flexneri produced small, opaque, convex, colorless and translucent colonies on Nutrient agar. The organism produced smooth, colorless and opaque colonies when growing on selective media Salmonella Shigella (SS) agar *Shigella flexneri* is a gram negative, nonmotile, rod shaped organism (Table.1). Further biochemical test was performed the observed and, the isolate was confirmed as *Shigella flexneri* (Table: 2). *Pseudomonas aeruginosa* is a gram negative rod shaped motile organism. The colonies were smooth, large, translucent, and low convex on nutrient agar. (Table-1). The colonies appear greenish, large and irregular non- fluorescent colonies on selective medium cetrimide agar. The results depicted in (Table.2) confirmed that the isolate belonged to *Pseudomonas aeruginosa*. The results were compared in accordance with the Bergey's manual of determinative bacteriology.

Identification of *Staphylococcus aureus*

Staphylococcus aureus is gram positive cocci, arranged in clusters. It is a nonmotile organism. *Staphylococcus aureus* produced circular, convex, golden yellow colonies with smooth shiny surface on nutrient agar. It grows with oil paint appearance in nutrient agar slant. It produced pink colored non-lactose fermenting colonies on Macconkey agar. It ferments mannitol and produced yellow colored colonies on mannitol salt agar. (Table. 1). The result revealed that the strain belonged to *Staphylococcus aureus.* The obtained results were compared with the Bergey's manual of determinative bacteriology. (Table-2).

Haemolytic activity pattern of the isolates

All the three isolates *Shigella flexneri*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* lysed the RBC cells present in the blood and produced a clear zone around their growth. All the isolates were found to be β- hemolytic and the results were depicted in (Table: 3, and Plate: 1).

Multidrug resistance patterns of the isolates

The isolates were evaluated for their resistance pattern against eleven antibiotics which were depicted in Table-4. In the present study, the isolate *Shigella flexneri* revealed resistance to Penicillin (10µg), Cefotaxime (30µg), Penicillin (2µg), Clotrimazole (25µg), Nalidixic acid

(30µg), Tobramycin (10µg), Ampicillin (10µg) and Vancomycin (30µg). Out of the eleven antibiotics tested, *Shigella flexneri* was found to be resistant to eight antibiotics. The results were exhibited in Plate: 2.

Similarly, the multidrug resistance pattern was studied for the isolate *Pseudomonas aeruginosa.* The result presented in (Table-5) revealed that the organism was found resistant to Penicillin (10µg), Cefotaxime (25µg), Levofloxacin (5µg), Penicillin (2µg), Clotrimazole (25µg), Streptomycin (10µg), Nalidixic acid (30µg), Tobramycin (10µg), Ampicillin (10µg) and Vancomycin (30µg). *Pseudomonas aeruginosa* was found resistant to ten antibiotics, out of the eleven antibiotics tested. (Plate: 3).

Simultaneously, *Staphylococcus aureus* was found to be resistant to Penicillin (10µg), Gatifloxacin (5µg), Cefotaxime (30µg), Penicillin (2µg), Clotrimazole (25µg) Streptomycin (10µg), Nalidixic acid (30µg), Tobramycin (10µg), Ampicillin (10µg) and Vancomycin (30µg). Out of the eleven isolates, screened *Staphylococcus aureus* revealed multidrug resistance against nine antibiotics. The above results suggested that all the three isolates were found to be Multidrug resistant strain (Table: 6 and Plate: 4).

MAR Index Analysis of the Isolates

The MAR index analysis revealed that all the three isolates had a very high MAR index value of >0.2. The MAR index values were ranged from 0.5-1.0. In the present study, *Pseudomonas aeruginosa* exhibited highest MAR value with 0.90 when compared to *Staphylococcus aureus* showed (0.81) and *Shigella flexneri* exhibited (0.72). (Table: 7).

Determination of minimum inhibitory concentration

Minimum inhibitory concentration is defined as the lowest concentration of a drug that will inhibit the visible growth of an organism after overnight incubation. In the present study, Minimum inhibitory concentration of amikacin against different concentration such as 128µg/ml, 64µg/ml, 32µg/ml, 16µg/ml, 8µg/ml, 4µg/ml, 2µg/ ml, 1µg/ml, 0.5µg/ml and o.25µg/ml were studied. Similarly the The result revealed that *Shigella flexneri* exhibited resistance to lower concentration of amikacin such as 2µg/mg to 0.25µg/ml (Table: 8 and Plate: 5).

Similarly, the minimum inhibitory concentration of *Pseudomonas aeruginosa* against different concentration of amikacin revealed resistance to 4µg/ml to 0.25µg/ml. (Table: 9, and Plate: 6). Whereas, *Staphylococcus aureus* revealed resistance to higher concentration of amikacin (32µg/ml) to lower concentration (0.25µg/ml). The resistivity pattern was depicted in (Table-10 and Plate: 7).

Plasmid Profile Studies of Multi Drug Resistant Strains in Agarose Gel Electrophoresis

Electrophoretic analysis of the plasmid DNA and plasmid profile of multidrug resistant bacteria was studied. Electrophoretic analysis of the plasmid DNA prepared was carried on 0.8%. agarose gel. Plasmid profile studied for multi drug resistant *Shigella flexneri*. Analysis of plasmid DNA revealed a single unique band with 1700 kbp. Similarly, Plasmid profiling was analyzed with multidrug resistant *Pseudomonas aeruginosa* by agarose gel electrophoresis. It was found out that *Pseudomonas aeruginosa* revealed single band with the size of plasmid exhibited 830kbp. The results were shown in (plate.20). *Staphylococcus aureus* exhibited single plasmid DNA on the basis of electrophoretic mobility the molecular size of plasmid DNA was calculated to be around 540kbp. 1kbp DNA was used as marker DNA. The results were exhibited in Plate:8.

PLATE :1. HAEMOLYTIC ASSAY OF MULTIDRUG RESISTANT ISOLATES ON BLOOD AGAR MEDIUM

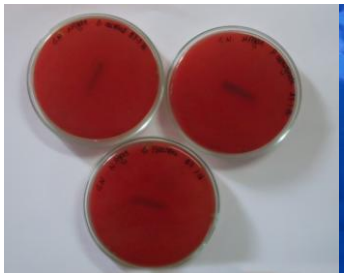

PLATE: 2. STUDIES ON MIC OF AMIKACIN TREATED AGAINST *Shigella flexneri*

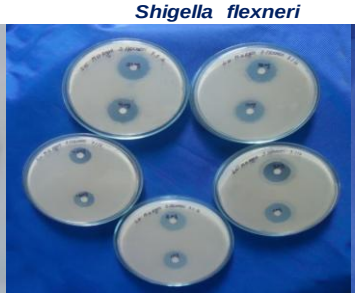

PLATE: 3. STUDIES OF MIC OF AMIKACIN TREATED AGAINST *Pseudomonas aeruginosa*

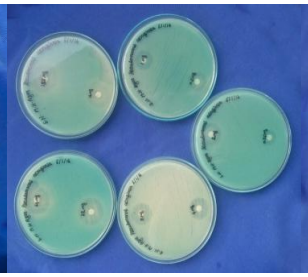

PLATE: 4. STUDIES ON MIC OF AMIKACIN TREATED AGAINST *Staphylococcus aureus*

PLATE: 5. EFFICACY OF DIFFERENT ANTIBIOTICS TO STUDY THE MULTIDRUG RESISTANCE AGAINST *Shigella flexneri*

PLATE: 4. EFFICACY OF DIFFERENT ANTIBIOTICS TO STUDY THE MULTIDRUG RESISTANCE AGAINST *Pseudomonas aeruginosa*

PLATE: 7. EFFICACY OF DIFFERENT ANTIBIOTIC TO STUDY THE MDR AGAINST *Staphylococcus aureus*

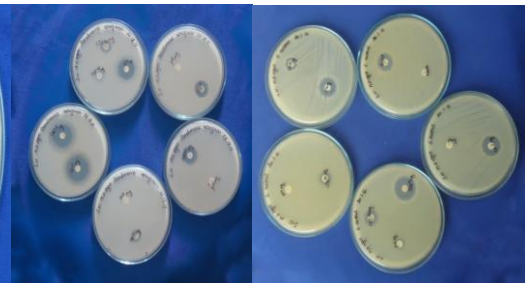

PLATE: 8. PLASMID PROFILE STUDIES OF MULTI DRUG RESISTANT STRAINS IN AGAROSE GEL ELECTROPHORESIS

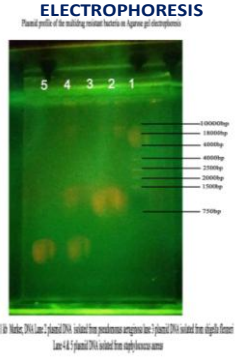

Table: 1: Morphological Characterization Methods of *Shigella flexneri Pseudomonas aeruginosa* and *Staphylococcus aureus*

Sr. No.	Morphological Characteristics	Observation		
		Shigella flexneri	***Pseudomonas aeruginosa***	***Staphylococcus aureus***
1.	**Colony Morphology**	On salmonella shigella (ss) agar the organisms produced smooth, opaque and colorless colonies.	On Cetrimide agar, the colonies appear greenish, not fluorescent, irregular large colonies.	On mannitol salt produced yellow colonies with yellow zone (mannitol ferments)
		On Nutrient agar, colonies are small, circular, convex, colourless and translucent	On Nutrient agar, in produced smooth, large, translucent low convex colonies.	On Macconkey agar, produced pink colonies.
		-	-	On Nutrient agar, produced circular, convex, golden yellow colonies with smooth shiny surface
2.	**Gram Staining**	Gram negative	Gram negative rod	Gram positive cocci in cluster
3.	**Motility**	Non-motile	Motile	Non-motile

Table: 2: Biochemical Characterization of *Shigella flexneri, Pseudomonas aeruginosa* and *Staphylococcus aureus*

Sr. No.	Biochemical Test	Observation		
		Shigella flexneri	***Pseudomonas aeruginosa***	***Staphyloccus aureus***
1.	**Indole Production Test**	Negative	Negative	Negative
2.	**Methyl Red Test**	Positive	Negative	Positive
3.	**Voges Proskauer Test**	Negative	Negative	Negative
4.	**Citrate Utilization Test**	Positive	Positive	Positive
5.	**Triple Sugar Iron Test**	Acid butt, acid Slant H_2S production	Alkaline slant With H_2S produced	Negative
6.	**Starch Hydrolysis Test**	Positive	Negative	Negative
7.	**Gelatin Hydrolysis Test**	Positive	Positive	Positive
8.	**Nitrate Reduction Test**	Positive	Positive	Positive

Table: 3: Haemolytic Activity *of Shigella flexneri, Pseudomonas aeruginosa* and *Staphylococcus aureus* on Blood Agar

Sr. No.	Microorganism	Haemolysis
1.	*Shigella flexneri*	β- hemolysis
2.	*Pseudomonas aeruginosa*	β – hemolysis
3.	*Staphylococcus aureus*	β- hemolysis

Table: 4: Multidrug Resistant Pattern of *Shigella flexneri* against Different Antibiotics

Sr. No.	Antibiotics	Zone of inhibition in millimeter	
		Shigella flexneri	**Resistance/Sensitivity pattern**
1.	Penicillin P^{10}µg	No zone	Resistant
2.	Gatifloxacin CTA^{5}µg	33	Sensitive
3.	Cefotaxime CTX^{30}µg	15	Resistant
4.	Levofloxacin LE^{5}µg	34	Sensitive
5.	Penicillin P^{2}µg	No zone	Resistant
6.	Co-Trimazole COT^{25}µg	No zone	Resistant
7.	Streptomycin S^{10}µg	24	Sensitive
8.	Nalidixic acid NA^{30}µg	17	Resistant
9.	Tobramycin TOB^{10}µg	17	Resistant
10.	Ampicillin A^{10}µg	No zone	Resistant
11.	Vancomycin VA^{30}µg	13	Resistant

Table: 5: Multidrug Resistant Pattern of Microorganism *Pseudomonas aeruginosa* treated against Different Antibiotics

Sr. No.	Antibiotics	Zone of Inhibition in Millimeter	
		Pseudomonas aeruginosa	**Resistance/ Sensitivity Pattern**
1.	Penicillin P^{10}µg	No zone	Resistant
2.	Gatifloxacin CTA^{5}µg	33	Sensitive
3.	Cefotaxime CTX^{30}µg	15	Resistant
4.	Levofloxacin LE^{5}µg	35	Resistant
5.	Penicillin P^{2}µg	No zone	Resistant
6.	Co-Trimazole COT^{25}µg	1	Resistant
7.	Streptomycin S^{10}µg	22	Resistant
8.	Nalidixic acid NA^{30}µg	18	Resistant
9.	Tobramycin TOB^{10}µg	18	Resistant
10.	Ampicillin A^{10}µg	No zone	Resistant
11.	Vancomycin VA^{30}µg	12	Resistant

Table: 6: Multidrug Resistant Pattern of Different Microorganism *Staphylococcus aureus* against Different Antibiotics

Sr. No.	Antibiotics	Zone of Inhibition in Millimeter	
		Staphylococcus aureus	**Resistant/ Sensitivity Pattern**
1.	Penicillin P^{10}µg	No zone	Resistant
2.	Gatifloxacin CTA^{5}µg	18	Resistant
3.	Cefotaxime CTX^{30}µg	16	Sensitive
4.	Levofloxacin LE^{5}µg	19	Sensitive
5.	Penicillin P^{2}µg	No zone	Resistant
6.	Co-Trimazole COT^{25}µg	15	Resistant
7.	Streptomycin S^{10}µg	16	Resistant
8.	Nalidixic acid NA^{30}µg	No zone	Resistant
9.	Tobramycin TOB^{10}µg	No zone	Resistant
10.	Ampicillin A^{10}µg	No zone	Resistant
11.	Vancomycin V^{30}µg	13	Resistant

Table: 7: Mar Index Resistance Pattern of *Shigella flexneri, Pseudomonas aeruginosa* and *Staphylococcus aureus*

Sr. No.	Microorganisms	MAR Index(A/B)	Resistance pattern
1.	*Shigella flexneri*	0.72	P^{10}µg, CTX^{30}µg, COT^{25}µg, NA^{30}µg, TOB^{10}µg, A^{10}µg, VA^{30}µg
2.	*Pseudomonas aeruginosa*	0.90	P^{10}µg, CTX^{30}µg, LE^{5}µg, COT^{25}µg, S^{10}µg, NA^{30}µg, TOB^{10}µg, A^{10}µg, VA^{30}µg
3.	*Staphylococcus aureus*	0.81	P^{10}µg, CTA^{5}µg, P^{2}µg, COT^{25}µg, S^{10}µg, NA^{30}µg, TOB^{10}µg, A^{10}µg, VA^{30}µg

Table: 8: Minimum Inhibitory Concentration of Amikacin against *Shigella flexneri* on Mueller Hinton Agar

Sr. No.	Different concentration of amikacin	Zone of inhibition (in millimeter)	
		Shigella flexneri	**Resistance/Sensitivity pattern**
1.	128µg	29mm	Sensitive
2.	64µg	27mm	Sensitive
3.	32µg	25mm	Sensitive
4.	16µg	23mm	Sensitive
5.	8µg	22mm	Sensitive
6.	4µg	20mm	Sensitive
7.	2µg	19mm	Resistant
8.	1µg	17mm	Resistant
9.	0.5µg	15mm	Resistant
10.	0.25µg	10mm	Resistant

Table: 9: Minimum Inhibitory Concentration of Amikacin against *Pseudomonas aeruginosa* on Mueller Hinton Agar

Sr. No.	Different Concentration of Amikacin	Zone of Inhibition (in millimeter)	
		Pseudomonas Aeruginosa	**Resistance/Sensitivity Pattern**
1.	128µg	26mm	Sensitive
2.	64µg	24mm	Sensitive
3.	32µg	22mm	Sensitive
4.	16µg	21mm	Sensitive
5.	8µg	20mm	Sensitive
6.	4µg	18mm	Resistant
7.	2µg	16mm	Resistant
8.	1µg	15mm	Resistant
9.	0.5µg	12mm	Resistant
10.	0.25µg	10mm	Resistant

Table: 10: Minimum Inhibitory Concentration of Amikacin against *Staphylococcus aureus* on Mueller Hinton Agar

Sr. No.	Different Concentration of Amikacin	Zone of Inhibition (in millimeter)	
		Staphylococcus aureus	**Resistance/Sensitivity Pattern**
1.	128µg	20mm	Sensitive
2.	64µg	19mm	Sensitive
3.	32µg	16mm	Resistant
4.	16µg	15mm	Resistant
5.	8µg	14mm	Resistant
6.	4µg	13mm	Resistant
7.	2µg	12mm	Resistant
8.	1µg	11mm	Resistant
9.	0.5µg	9mm	Resistant
10.	0.25µg	10mm	Resistant

DISCUSSION

The resistance to the antimicrobials has increased over the years and normal intestinal microbial flora becomes a reservoir for resistant genes (Okeke *et al.*, 2000). This may be due to an inevitable genetic response to the strong selectivity pressure imposed by antimicrobial chemotherapy, which plays a vital role in the evolution of antibiotic resistance among

bacteria. The bacteria then pass the plasmid containing resistant gene among other bacterial cells and species (Chakraborty, *et al.*, 2011).

Multidrug Resistant has become a common feature of many microorganisms especially the human pathogens that hurdles chemotherapy. To overcome this problem, it is obligatory to identify the Multidrug Resistance (MDR) character of an isolate.

Diarrhoea is a significant health problem worldwide especially in the developing countries where adequate sanitation facilities were lacking. *Shigella flexneri* is notorious for producing not only large scale epidemics but also pandemics which are characterized by multidrug resistance. The main study of treatment is appropriate antibiotics but development of drug resistance poses a serious therapeutic challenge (Dipika *et al.*, 2004).

Pseudomonas aeruginosa has a particular propensity for the development of resistance. It is naturally resistant to many antibiotics because of its relatively impermeable outer membranes and it can also easily acquire scenarios.

Outbreaks of multidrug-resistant *Pseudomonas aeruginosa* colonization or infection have been reported on urology wards, a burn unit, hematology/oncology unit, and adult and neonatal critical care unit (Jesudasan, et al., 1985). Various medical devices and environmental reservoirs have been implicated in these outbreaks, including antiseptic solutions and lotion; endoscopy equipment; ventilator apparatus; and mouth swab.

Over the past decade, there has been an increase in the rate of infection and disease caused by *Staphylococcus aureus* particularly MRSA throughout the world (Sadaka, *et al.*, 2009). *Staphylococcus aureus* is major cause of nosocomial infection leading to a wide range of diseases including endocarditis, osteomyelitis, toxic shock syndrome, pneumonia, food poisoning and carbuncles. In India, hospital MRSA is responsible for 30-80% of hospital acquired infection (Nadig, *et al.*, 2006).

The present investigation was aimed to isolate the multidrug resistant *Shigella flexneri*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and its antibiotic resistant profile were studied. The clinical samples such as stool and urine samples were obtained from the Government hospital, Madurai and samples were plated on different selective agar medium in order to isolate *Shigella flexneri, Pseudomonas aeruginosa* and *Staphylococcus aureus.* All the three isolates were identified by standard techniques.

In the present study, hemolytic activity of the clinical isolates was performed by inoculating them onto the blood agar plates. The blood which contains the RBC which was lysed by the presence of hemolysis was determined by the formation of zone of α, β and γ hemolysis. All the three isolates were found to be β- hemolytic.

Multidrug resistance was defined as resistance to three or more antibiotics. The isolates were evaluated for their resistance pattern against eleven antibiotics. *Shigella flexneri,* was resistant to eight antibiotics *Pseudomonas aeruginosa* was found to be resistant to ten antibiotics and *Staphylococcus aureus* revealed resistance to nine antibiotics. It was found out that *Shigella flexneri* was found to be sensitive to Gatifloxacin, Levofloxacin and Streptomycin. Our work coincides with the result of (Jenifer Burn Peter, 1985). *Shigella flexneri* was found resistant to ampicillin, nalidixic acid and cotrimoxazole. Our result is in total conformity with the work of (Dutta, *et al.*, 2003).

Simultaneously, the multidrug resistant pattern of *Pseudomonas aeruginosa* was found resistant to Streptomycin, Cotrimoxazole, Cefotaxime, Penicillin, Ampicillin, Vancomycin, Nalidixic acid and Tobramycin. Similar results were observed by the work of (Paranjothi and Deepa 2004). In which they emphasized that *Pseudomonas aeruginosa* was found resistant against Streptomycin, Cotrimoxazole and Cefotaxime.

Similarly, the multidrug resistant pattern of *Staphylococcus aureus* was studied. *Staphylococcus aureus* revealed resistance to Gatifloxacin, Penicillin, Clotrimazole, Streptomycin, Nalidixic acid Tobramycin, Ampicillin and Vancomycin. Vancomycin was recommended as an active drug against *Staphylococcus aureus* (Marais *et al.*, 2009). But in present study, *Staphylococcus aureus* revealed resistance to Vancomycin. Our result coincides with the findings of Campanile *et al.*, 2009 and Samie and Shivambu, 2011. Where in, they emphasized found that *Staphylococcus aureus* was found resistance to vancomycin, nearly 14%, further they reported that the level of resistant was high. It was presumed that biofilm production appeared to be a factor for the increased antibiotic resistance as well as pathogenicity in these strain.

The MAR index analysis revealed that all the three bacterial isolates had a very high MAR index value of >0.2. Bacteria having MAR index > 0.2 originate from an environment where several antibiotics are used (Tambekar *et al.,* 2006). Our results are in total agreement with the work of (Subramain and Vignesh 2012).

Minimum inhibitory concentration (MIC) is used to determine the concentration of antibiotics at which there is no visible growth of the organism (Jennifer, 2001). The Minimum Inhibitory Concentration value was found in line with the corresponding resistance or sensitive pattern. The Minimum Inhibitory Concentration of selected *Shigella flexneri, Pseudomonas aeruginosa* and *Staphylococcus aureus* revealed resistance to amikacin. The value of Minimum Inhibitory Concentration for Amikacin ranged from 128µg/ml to 0.25µg/ml. The high Minimum Inhibitory Concentration (MIC'S) could have a profound effect on the treatment of the diseases. (Lari, *et al.*, 2000). Our work was in total conformity with the work of Ashkenazi *et al.* suggested that resistance to amikacin was developed among *Shigella flexneri* strains.

In the present study, the isolation of plasmid DNA was performed for *Shigella flexneri* which revealed the presence of the plasmid of the size of 1700 kbp. Multidrug resistant bacterial pathogens are the serious problem nowadays faced by the clinicians. Such a multiple resistant strains enter the community and hybridize with non-MDR strains resulting in the transfer of resistant plasmids and become a serious problem in controlling these strain. Similar work was carried by Estahbanati, et al., 2002.

In the present study, the plasmid profile analysis was studied for multidrug resistant *Pseudomonas aeruginosa.* The result revealed that the bacteria transfer its resistance through plasmid. *Pseudomonas aeruginosa* possess plasmid through which resistant traits of bacteria can be exchanged between one organism to another. Our work coincides with the finding of Silva, *et al.,* 2003) in which they studied the plasmid profile pattern of *Pseudomonas aeruginosa.*

The isolation of plasmid was performed using agarose gel electrophoresis and observation under UV- transilluminator show the single band for the *Staphylococcus aureus* with the molecular weight of plasmid with 540 kbp. This result agreed with the findings of Al-Holder, et al., 1995 wherein they emphasized that *Staphylococcus aureus* produced single prominent single prominent band with the molecular weight of plasmid ranging from 21-22 kbp. Plasmid isolation procedure is based on the fact that plasmids occur in the supercoiled form within the host cells. After gentle cell lysis, all intracellular macromolecules have to be eliminated whereas plasmid DNA is enriched and purified (Gillespi, et al., 2000).

The present study revealed that plasmid was randomly distributed in multidrug resistant strain. The genes for multidrug resistance might be located on plasmid DNA or chromosomal DNA. There was a consistent relationship was observed between antimicrobial resistance and plasmid profile.

CONCLUSION

The resistance to the antimicrobials has increased over the years, which may be due to an inevitable genetic response to the strong selectivity pressure imposed by antimicrobial chemotherapy, which plays a vital role in the evolution of antibiotic resistance among bacteria. The present investigation was carried out to isolate and to identify the *Shigella flexneri, Pseudomonas aeruginosa* and *Staphylococcus aureus* from clinical samples obtained from Government hospital, Madurai. The different clinical specimens were plated on different agar media such as Nutrient agar, *Salmonella shigella* (ss) agar, Cetrimide agar, Mannitol agar and Macconkey agar in order to isolate *Shigella flexneri, Pseudomonas aeruginosa* and *Staphylococcus aureus.* The three isolates were subjected to hemolytic activity and the zone was measured. Haemolytic activity of *Shigella flexneri, Pseudomonas aeruginosa* and *Staphylococcus aureus* were studied in order to ascertain the virulence factor such as a hemolysis, cytolysin and enterotoxins by blood agar medium. The isolates were evaluated for their resistance pattern against eleven antibiotics. The zone of inhibition produced by the organisms was compared with the standard interpretative chart in order to find out their resistant pattern. The present study *Shigella flexneri* was resistant to such as Penicillin, Cefotaxime, Clotrimazole, Nalidixic acid, Tobramycin, Ampicillin, and Vancomycin. Similarly, *Pseudomonas aeruginosa* was found to be resistant to ten antibiotics. In the present study, Minimum Inhibitory Concentration of amikacin against different concentration such as 128µg/ml, 64µg/ml, 32µg/ml, 16µg/ml, 4µg/ml,1µg/ml,0.5µg/ml, and 0.25µg/ml. were studied. *Shigella flexneri* exhibited resistance to lower concentration of amikacin such as 2µg/ml. to 0.25µg/ml similarly, *Pseudomonas aeruginosa* revealed resistance to 4µg/ml to (0.25µg/ml). Whereas, *Staphylococcus aureus* revealed resistance to higher concentration of amikacin 32µg/ml to lower concentration (0.25µg/ml). Plasmid profile studied was for multidrug resistant organisms analysis of plasmid DNA revealed a single unique band with 1700 kbp for *Shigella flexneri*, *Pseudomonas aeruginosa* revealed single band with the size of plasmid exhibited 830 kbp. The molecular size of plasmid DNA was calculated to be around 540 kbp for *Staphylococcus aureus.*

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