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EVALUATION OF *IN VITRO* ANTIMICROBIAL ACTIVITY OF NEW GENERATION POWERFUL DISINFECTANTS

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ABSTRACT

Disinfectants were tested for their inhibitory activities against commonly occurring bacterial strains *Acinetobacter baumannii, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus and fungal strains like Candida albicans* and *Aspergillus niger*. The inhibition was recorded by measuring the zone of inhibition of the disinfectants on the tested organisms. The results revealed that the disinfectants Sepsonil DX Cream**,** Sarvodin, Povidone Iodine 7.5% Solution USP, Sterital, Sidol + (Cetrimide and Chlorohexidine Gluconate), Sidol, Sterilhandz, Sepsonil DX, Microcleer + Chlorhexidine Surgical Wash and Povidone-Iodine Cleaning Solution U.S.P. 10% w/v Surgical Scrub were very effective as it inhibited the growth of *Acinetobacter baumannii* (BAA-1605), Methicillin resistant *Staphylococcus aureus* (ATCC 43300), *Escherichia coli* (ATCC 25922, ATCC 8739, BAA196, BAA198, BAA199, BAA 200, BAA 201 and BAA 457), *Pseudomonas aeruginosa* (BAA-2109), *Klebsiella pneumonia* (ATCC 51503), *Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 9027). Only disinfectants Microcleer + Chlorhexidine Surgical Wash and Povidone-Iodine Cleaning Solution U.S.P. 10% w/v Surgical Scrub were resulted antifungal activity against *Candida albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 16404).

KEYWORDS: Carbapenem resistant *K. pneumonia* (CRKP), Methicillin resistant *Staphyloccous aureus (MRSA),* resistance, antimicrobial, novel disinfectant.

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INTRODUCTION

Antiseptics and disinfectants are extensively used in hospitals, health care and research institutes on a variety of applications. The number of deaths associated with the germ like methicillin-resistant Staphylococcus aureus(MRSA) would exceed those attributed to HIV-AIDS, Parkinson's disease, emphysema or homicide each year. By extrapolating data collected in nine places (globally/within India), the researchers estimated that 94,360 patients developed an invasive infection from the pathogen in 2005 and that nearly one of every five or 18,650 of them died due to infection with MRSA. The study points out that it is not always possible to determine whether a death is caused by MRSA or merely accelerated by it[1]. Table below indicates the available data on difference in death rates (mortality) between resistant and sensitive bacteria[2].

Bacteria	Death rate	
	Resistant strain	Sensitive strain
E.coli	32.0%	17.0%
A.baumannii	16.4%	5.4%
A.baumannii	53.8%	31.0%*
K.pneumoniae	42.9% (CRKP)	18.9%
K.pneumoniae	43.8% (CRKP)	12.5%
K.pneumoniae	38.0%	12.0%
S.aureus	36.4%(MRSA)	27.0%
S.aureus	23.6%(MRSA)	11.5%

Comparison of death rates (mortality) in patients with resistant or sensitive strains of bacteria. *=not fully sensitive.

Over the year, disinfectants have played a major role in the replacement of antimicrobial in controlling infectious diseases. The effective antimicrobial properties of the disinfectants are presumed to be influenced by their formulation, intrinsic property of Synergism/additive effects on the microbes and organic load, temperature and dilution rate and test methods[4, 5]. Resistance of micro organisms to vast chemicals has become a concern in the investigation of new drug and disinfectants in controlling the load of pathogenic micro organisms. Although the precise origin of such resistance remains unclear, different studies have shown that it is a multifactorial process involving the spatial organization of the biofilm and continuation mutation of the strains. There is increasing evidence that the disinfectant resistance of pathogenic bacteria grown in laboratory cultures may differ greatly from that of the same species occurring in the environment. As an advent of formulation of new disinfectants and antiseptics, Sarvotham Care Ltd., has introduced number of products which were analyzed for antimicrobial property by Agar diffusion method against various disinfectant resistant and pathogenic bacteria such as methicillin resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii.* Common human isolates such as various serotypes of *Escherichia coli*, *Klebsiella pneumonia* and *Staphylococcus aureus* were tested as tester strains to evaluate the efficacy of the disinfectants. Further; commonly available fungal strains like *Candida albicans* which is being a common cause of urinary tract infection and *Asperigillus niger* a potent source of food poisoning produces potent mycotoxins called ochratoxins[7] were also involved in the investigationto understand the anti microbial proency of different formulations developed by Sarvotham Care Ltd., .

MATERIALS AND METHODS

(i) Disinfectants

Sepsonil DX Cream**,** Sarvodin, Povidone Iodine 7.5% Solution USP, Sterital, Sidol+(Cetrimide and Chlorohexidine Gluconate), Sidol, Sterilhandz, Sepsonil DX, Microcleer+Chlorhexidine Surgical Wash and Povidone-Iodine Cleaning Solution U.S.P. 10% w/v Surgical Scrub, supplied by Sarvotham Care Ltd, 1-20-248, Umajay Complex, 1st Floor Rasoolpura, Secunderabad – 500003.

(ii) Tester Strains

Acinetobacter baumannii (BAA-1605), Methicillin resistant *Staphylococcus aureus* (ATCC 43300), *Escherichia coli* (ATCC 25922), *Escherichia coli* (BAA196), *Escherichia coli* (BAA198), *Escherichia coli* (BAA199), *Escherichia coli* (BAA 200), *Escherichia coli* (BAA 201), *Escherichia coli* (BAA 457), *Pseudomonas aeruginosa* (BAA-2109), *Klebsiella pneumonia* (ATCC 51503), *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), *Candida albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 16404) were procured from American type culture collection (ATCC) USA.

(iii) Media Preparation

Dehydrated Medias such as Sabouraud Dextrose Agar with Antibiotics (Agar Medium C) and Casein Soyabean Digest Agar were procured from Himedia Laboratories and prepared as per manufacturer's instruction.

(iv) Inoculum Preparation

Each bacterial tester strains were grown for 24 hrs on Casein Soyabean Digest Agar, were resuspended in Phosphate Buffer Saline of pH 7.2±0.2 and diluted to 0.5 McFarland tubes (inoculums with concentration of approximately 10^8 Colony Forming Units). *C.albicans* was grown for 48 hrs on Sabouraud Dextrose Agar with Antibiotics (Agar Medium C) and inoculums was adjusted similar to bacterial cultures. *A. niger* was grown for 5-7 days on Sabouraud Dextrose Agar with Antibiotics (Agar Medium C) and spores were collected to medium with tween 80 and spore concentration was adjusted to obtain approximately 10^8 Colony Forming Units. The inoculums concentrations were determined by serial dilution and stored at 2 to 8 ºC before use.

(v) Evaluation of Antimicrobial activity

Volume of 0.1 mL of each disinfectant was introduced on to the centre of the plate swabbed with respective tester strain. Bacterial cultures were inoculated on Casein Soyabean Digest Agar and incubated at 37±1ºC for 24-48 hrs, yeast and mould was inoculated on Sabouraud Dextrose Agar with Antibiotics and incubated at 25±2ºC for 48 hrs and 5-7 days respectively. The zone of inhibition was determined by measuring the diameter in millimeters of zone to which the disinfectant inhibited the growth of the organism.

RESULTS

Table 1
Activities of disinfectants against Escherichia coli

Disinfectants	Tester Strains - *Escherichia coli*							
	ATCC 25922	BAA 196	BAA 198	BAA 199	BAA 200	BAA 201	BAA 457	ATCC 8739
Sepsonil DX cream	0.5	0.5	0.4	0.4	0.3	0.6	0.5	0.7
Sarvodin	0.4	0.4	0.5	0.5	0.5	0.3	0.5	0.8
Povidone Iodine 7.5% Solution USP	0.5	0.4	0.7	0.4	0.6	0.4	0.7	0.8
Sterital	0.4	0.5	0.6	0.5	0.7	0.4	0.8	0.6
Sidol + (Cetrimide and Chlorohexidine Gluconate)	0.5	0.5	0.9	0.9	0.4	0.5	0.8	0.8
Sidol	0.2	0.3	0.4	0.6	0.4	0.4	0.9	0.4
Sterilhandz	0.9	0.9	0.7	0.8	0.4	0.3	0.4	0.8
Sepsonil DX	0.6	0.9	0.7	0.9	0.6	0.3	0.8	0.9
Microcleer +Chlorhexidine Surgical Wash	0.6	0.7	0.6	0.7	0.9	0.7	0.8	0.8
Povidone-Iodine Cleaning Solution U.S.P. 10% w/vSurgical Scrub	0.5	0.6	0.8	0.7	0.7	0.6	0.7	0.6

Table 2
Activities of disinfectants against other bacteria

Disinfectants	Tester Strains					
	Acinetobacter baumannii (BAA-1605)	Methicillin Resistant *Staphylococcus aureus* (ATCC 43300)	*Staphylococcus aureus* (ATCC 6538)	*Pseudomonas aeruginosa* (ATCC 2109)	*Pseudomonas aeruginosa* (ATCC 9027)	*Klebsiella pneumoniae* (ATCC 51503)
Sepsonil DX cream	0.8	0.3	0.4	0.4	0.3	0.4
Sarvodin	0.9	0.8	0.7	0.4	0.5	0.4
Povidone Iodine 7.5% Solution USP	0.7	0.6	0.5	0.8	0.8	0.8
Sterital	0.5	0.5	0.5	0.6	0.5	0.5
Sidol + (Cetrimide and Chlorohexidine Gluconate)	0.8	0.9	0.8	0.6	0.7	0.2
Sidol	0.9	0.8	0.5	0.3	0.7	0.5
Sterilhandz	0.5	0.9	0.5	0.4	0.4	0.9
Sepsonil DX	0.9	0.9	0.6	0.7	0.6	0.8
Microcleer + Chlorhexidine Surgical Wash	0.7	0.7	0.8	0.7	0.7	0.7
Povidone-Iodine Cleaning Solution U.S.P. 10% w/v Surgical Scrub	0.9	0.9	0.6	0.6	0.7	0.4

Table 3
Activities of disinfectants against yeast and mould

Disinfectants	**Tester Strains**	
	Candida albicans (ATCC 10231)	*Aspergillus niger* (ATCC 16404)
Sepsonil DX cream	0.5	0.0
Sarvodin	0.6	0.0
Povidone Iodine 7.5% Solution USP	0.5	0.0
Sterital	0.6	0.0
Sidol + (Cetrimide and Chlorohexidine Gluconate)	0.6	0.0
Sidol	0.5	0.0
Sterilhandz	0.5	0.0
Sepsonil DX	0.5	0.0
Microcleer + Chlorhexidine Surgical Wash	0.7	0.4
Povidone-Iodine Cleaning Solution U.S.P. 10% w/v Surgical Scrub	15	12

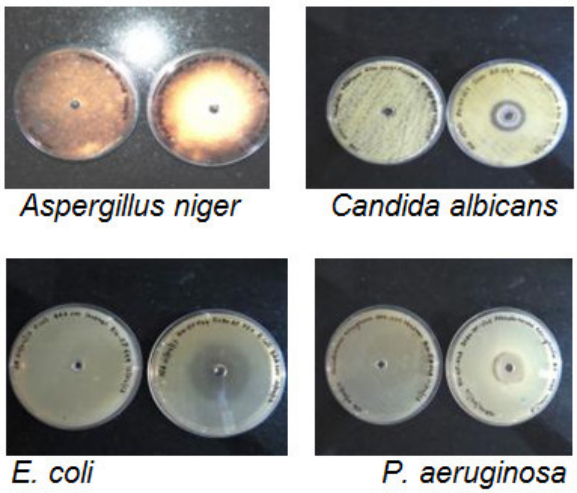

Figure 1
Images indicating antimicrobial activity against specific micro organism

DISCUSSION

The results obtained have revealed that; the antimicrobial activities of the tested disinfectants were effective against various micro organisms. Table 1 indicates the inhibitory activity of different disinfectants against various strains of *Escherichia coli.* Virulent strains of *E. coli* which are known to cause gastroenteritis, urinary tract infections, and neonatal meningitis. In rare cases, virulent strains are also responsible for hemolytic-uremic syndrome, peritonitis, mastitis, septicemia and Gram-negative pneumonia[16]. UPEC (Uropathogenic *E. coli*) is one of the main causes of urinary tract infections. Among all the tester strains *E. coli* BAA 457 is a causative agent of Urinary Tract Infection (UTI). Among all the disinfectants tested Sidol has shown a mild activity against all the strains of *Escherchia coli*. Sidol+ (Cetrimide and Chlorohexidine Gluconate), Sterilhandz, Sepsonil DX, Microcleer+Chlorhexidine Surgical Wash and ovidone-Iodine Cleaning Solution U.S.P. 10% w/v Surgical Scrub has shown major activity in inhibiting the *Escherichia coli* strains. Table 2 illustrates activities of disinfectants against other bacteria including drug resistant *Staphylococcus aureus* and *Acinetobacter baumannii*. Among the disinfectants tested, all the disinfectants had shown inhibitory activity against *Acinetobacter baumannii,* Gram negative coccobacilli, an opportunistic pathogen in humans affecting people with compromised immune systems, very important nosocomial infection. Prevention of *Acinetobacter baumannii* in hospitals focuses on increased hand washing frequently and more periodic diligent sterilization procedures.

Methicillin resistant *Staphylococcus aureus* (MRSA) is a bacterium responsible for several illness, which are difficult-to-treat infections in humans. MRSA is troublesome in hospitals, prisons and nursing homes, where patients with open wounds, invasive devices. Immunocompromised patients are at greater risk of infection than the general public. Among the disinfectants tested, except for Sepsonil DX cream, all other disinfectants has shown inhibitory activity being a solution for removal of resistant bacteria. *Pseudomonas aeruginosa* can cause disease in animals and humans. The organism infects the tissues and those with immune compromised patients..The organism is also found in and on medical devices, can cause cross-infections in hospitals and clinics. Biofilm of *P. aeruginosa* can cause chronic opportunistic infections, which are a serious problem for medical care in industrialized societies, especially for immunocompromised patients and the elderly. They often cannot be treated effectively with traditional antibiotic therapy. Povidone Iodine 7.5% Solution USP, Sidol+ (Cetrimide and Chlorohexidine Gluconate), Sepsonil DX, Microcleer+Chlorhexidine Surgical Wash and Povidone-Iodine Cleaning Solution U.S.P. 10% w/v Surgical Scrub are found to be solution in inhibiting the *Pseudomonas aeruginosa* (as per the results indicated by the tester strains).

Klebsiella pneumoniae although found in the normal flora of the mouth, skin and intestines, it can cause destructive changes to human lungs if aspirated. It is clinically the most significant member known to induce pneumonia. Among the disinfectants, Sidol+ (Cetrimide and Chlorohexidine Gluconate) resulted in minimum inhibitory activity against *Klebsiella pneumoniae*. Table 3 illustrates the inhibitory activity against *C. albicans* and *A. niger*. *Candida albicans* grows as both yeast and filamentous cell and is a opportunistic agent of oral and genital infection in humans.. Povidone-Iodine Cleaning Solution U.S.P. 10% w/v Surgical Scrub was found to result in maximum inhibitory activity against *C. albicans*.

Amongst all other disinfectants tested only Microcleer+Chlorhexidine Surgical Wash and Povidone-Iodine Cleaning Solution U.S.P. 10% w/v Surgical Scrub showed inhibitory activity against *A. niger*.

CONCLUSION

The main objective of this study is to compare the efficiency of new generation disinfectants and to introduce the same against the world of infectious resistant and

non resistant micro organisms. The following conclusions were obtained.

a. Among the entire new generation disinfectants tested in this study, all the products had the efficiency against all the cultures tested, except few disinfectants against *A.niger*.
b. Microcleer + Chlorhexidine Surgical Wash and Povidone-Iodine Cleaning Solution U.S.P. 10% w/v Surgical Scrub had a better disinfection effect on *C. albicans* and *A. niger*.
c. Use of new generation disinfectants, introduced by Sarvotham Care Ltd to the medical world will bring about changes in eradicating resistant micro organisms and spread of nosocomial infection and promising reduction in the mortality rates due to microbial infection.

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