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Isolation and Characterization of Microbes from Contaminated Kitchen Vassals and Analysis their Antibiotic sensitivity

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ABSTRACT

Pathogenic organisms are continuously entering the home with foods (foodborne) or through water (waterborne), through foods prepared in the home by an infected person (person-to-person spread), through the air (airborne), by insects or via pets. In the present study was isolation and identification of microorganisms from the kitchen samples of contaminated area and analysis Antibacterial activity of isolated organisms against different commercial antibiotics. In this present study the five types of bacterial colonies were identified such as Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Serratia marcescens and Staphylococcus aureus. Aspergillus niger, Aspergillus flavus, Candida albicans and Penicillium were isolated from the contaminated kitchen environment.

Keywords: *Bacteria, Fungi, Contaminated Kitchen and Antibiotic Sensitivity.*

INTRODUCTION

Studies have shown that many sites in the kitchen become contaminated when food harbouring indicator bacteria or *Salmonella* spp. is prepared (de Wit *et al.,* 1979; Zhao *et al.,* 1998). In the domestic environment, the kitchen is particularly important in spreading infectious diseases. (Bryan, 1988) indicated that a colonized person handling the implicated food was the most frequently identified factor that contributed to staphylococcal food poisoning, shigellosis and typhoid fever. Several studies on bacterial contamination in the kitchen were carried out in the past decades (Finch *et al.* 1978; Speirs *et al.,* 1995). Bacterial load of hand towels, dishcloths, tea towels, steel sinks and working surfaces were implicated to be the frequent sites (Finch *et al.,* 1978; Borneff *et al.,* 1988; Josephson *et al.,* 1997; Ikawa and Rossen, 1999; Kusumaningrum *et al.,* 2002). Some other bacterial infections associated with contaminated kitchen environment are caused by *Campylobacter, Listeria, Staphylococcus aureus, Bacillus cereus* and *Escherichia coli* (Dufrenne *et al.,* 2001; Regnath *et al.,* 2004). Fungi such as *Aspergillus niger* has also been implicated to cause heavy environmental contamination in the kitchen (London *et al.,* 1996). Keeping the above view in mind the present work has been carried out Isolation and Identification of microorganisms (Bacteria and Fungi) from the kitchen samples of contaminated area and Antibacterial activity of isolated organisms against different commercial antibiotics.

MATERIALS AND METHODS

Collection of Kitchen Samples:

In this study 25 kitchen samples of contaminated area were collected from Thanjavur district (Plate - I and **Table 1**). The samples were collected in sterile, dry, wide necked, leak proof container. If immediate delivery to the laboratory is not possible, the should be refrigerated at 4ºC.

Isolation Identification Microorganisms:

Bacterial and fungal spices were isolated from the collected sample using nutrient agar and Potato dextrose agar by serial dilution plate method. The samples serially diluted from 10^{-1} to 10^{-7} with 0.85% saline. The morphological and biochemical characteristics were conducted by Norris and Ribbons, (1972) methods for identify the bacteria.

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Antimicrobial Activity:

The antibiotic sensitivity of isolated microbial species to the commercial antibiotic tests was analyzed by disc diffusion (Kirby-Bauer) method. Antimicrobial activity test was carried out following the modification of the method originally described by Bauer *et al.,* (1996). The results obtained in the present investigation were subject to statistical analysis like Mean ($\bar{x}$) and Standard Deviation (SD) by Zar (1984).

RESULTS AND DISCUSSION

The present study totally five different bacterial species were observed after 24 hrs incubation. The isolated bacterial colonies are named as CKB1, CKB2, CKB3, CKB4 and CKB5. Similarly four different fungal species was observed after 72hrs incubation the isolated fungi are named as CKF1, CKF2, CKF3 and CKF4. The morphological characteristics and biochemical tests of Gram positive and Gram negative bacteria were studied and the results were compared with Bergey's manual of systemic bacteriology. The ability of Gram positive and Gram negative bacteria to ferment different carbohydrate was also noted **Table 1**. During microbiological studies five types of bacterial colonies were identified such as *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Serratia marcescens* and *Staphylococcus aureus* respectively (**Table 2** and **Fig. 1**). Similarly Domestic kitchen environment are potential places for harboring and spreading pathogenic bacteria including *Pseudomonas spp. Bacillus spp, Paenibacillus spp., Micrococcus spp., Acinetobacter spp., Salmonella spp.* according to Kusumaningrum *et al.,* (2002); Tumwine *et al.,* (2003); Borneff *et al.,* (1985,1989) these pathogen survive on the surface for hours or days, depending on the species.

Aravenitidou *et al.,* (2000) isolated *Aspergillus* and *Penicillium* species as well as *Candida* from the water, treated water and dialysis solution samples. In this present study the *Aspergillus niger, Aspergillus flavus, Candida albicans* and *Penicillium* were isolated from the contaminated kitchen environment. The only one type of yeast like fungi (*Candida albicans*) present in the three infected sample. *Aspergillus niger* was present in five infected samples. Whereas the *Aspergillus flavus* and *Penicillim sp* were found in four cases.

In the present study Pipercillin have maximum antibacterial activity against all bacterial pathogens when compared to other antibiotics. At the same time minimum inhibitory activity observed against *Escherichia coli*. The medium level of antimicrobial activity was shown by Erythromycin and Tetracycline. Among the isolated bacteria maximum growth suspension were observed in *Klebsiella pneumoniae*. Whereas minimum range of growth suppression was showed by *Escherichia coli.* The Erythromycin was effective against Gram positive bacteria isolated in the present study. Kapoor and Aggarwal, (1997) illustrated more than 50% of *Escherichia coli* shows sensitivity to

Amikacin, Ciprofloxacin and Gentamicin. Resistance to seen to Tetracycline and Ampicillin, which correlates with Obi *et al.*, (1996). In this present study the *E. coli* is sensitivity to Gentamicin, Tetracycline, Piperacillin and Rifampicin (**Table 3** and **Fig. 2**).

Totally four commercial antibiotics were tested against fungal isolates and the results were represented in **Table 4** and **Fig. 3**. In this study highest antifungal activity were noted Clotrimazole against all fungal pathogens. The medium level antifungal activity was shown by Nystatin and Amphotericin-B. A low level of antifungal activity was shown by Ketaconazole. This has no activity against *Aspergillus niger, Candida albicans* and *Penicillin spp.* Gupta, (2001) reported Amphotericin-B is resistant to *candida* and *Aspergillus*. It's coincides with the present study that clotrimazole in highest antifungal activity against *Aspergillus flavus, Aspergillus niger, Candida albicans* and *Pencillium sp.*

Table No. 1: Biochemical test for Bacterial identification

S. No	Isolated organisms	Gram staining	Motility	Shape	Indole	Methyl red	VP	Citrate	Urease	TSI	Catalase	Oxidase	Carbohydrate fermentation		
													Lactose	Dextrose	Sucrose
1.	CKB1	-	+	rod	+	+	-	-	-	A/A	+	-	AG	AG	AG
2.	CKB2	-	-	rod	-	-	+	+	-	A/A	+	-	AG	AG	AG
3.	CKB3	-	+	rod	-	-	-	+	+/-	K/K	-	+	-	A	-
4.	CKB4	-	+	rod	-	-/+	+	+	-	K/A	+	-	-	AG	AG
5.	CKB5	+	-	cocci	-	+	+	-	-	K/A	+	-	A	A	A

+ - positive; - - Negative; ± - variable; A - Acid; B - A/A – Acid slant and acid butt; K/K - Alkaline slant and alkaline butt; K/A - Alkaline slant Acid butt

Table No. 2: Percentage of Isolated Bacteria

S. No.	Name of organisms	Number of Sample	Percentage
1	***Escherichia coli***	7	28
2	***Klebsiella pneumoniae***	4	16
3	***Pseudomonas aeruginosa***	5	20
4	***Serratia marcescens***	3	12
5	***Staphylococcus aureus***	6	24

Table No. 3: Antibacterial Activity

S. No.	Antibiotics	Zone of inhibition mm in diameter				
		Escherichia Coli	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Serratia marcescens*	*Staphylococcus aureus*
1	**Erythromycin**	-	18	7	12	15
2	**Vancomycin**	-	12	8	-	11
3	**Rifampicin**	7	8	7	7	9
4	**Piperacillin**	8	11	15	18	11
5	**Norfloxacin**	-	8	11	12	10
6	**Tetracycline**	9	16	7	13	17
7	**Clindamycin**	-	9	-	8	10
8	**Streptomycin**	-	15	12	12	10
9	**Ampicillin**	-	7	7	11	7
10	**Gentamicin**	7	10	-	7	11

Table No. 4: Antifungal Activity

S. No.	Antibiotics	Zone of inhibition mm in diameter			
		Aspergillus flavus	*Aspergillus niger*	*Candida albicans*	*Penicillium sp.*
1	**Amphotericin- B**	17	11	11	10
2	**Clotrimazole**	15	17	15	17
3	**Ketoconazole**	8	-	-	-
4	**Nystatin**	14	16	14	15

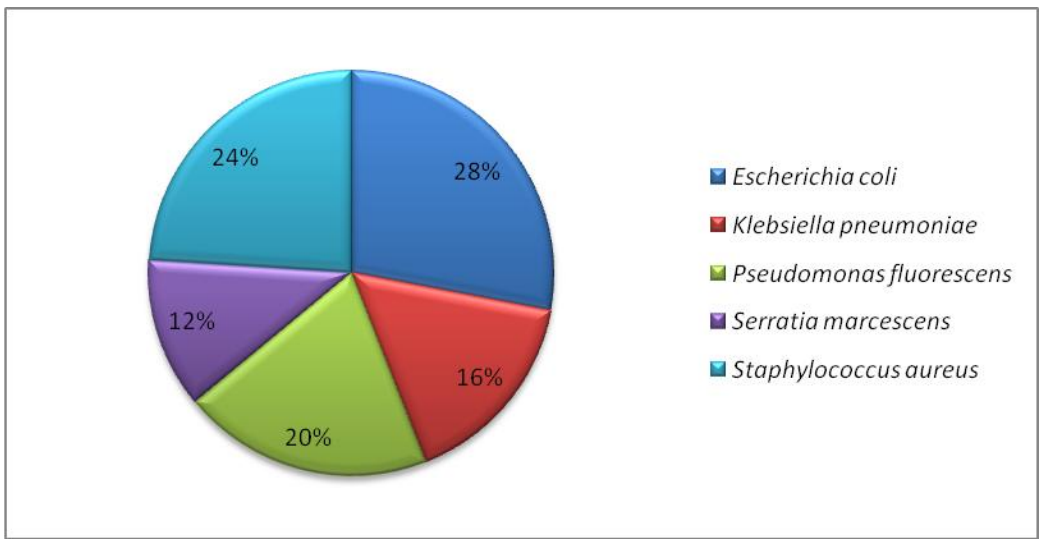

Fig. 1: Percentage of Isolated Bacteria

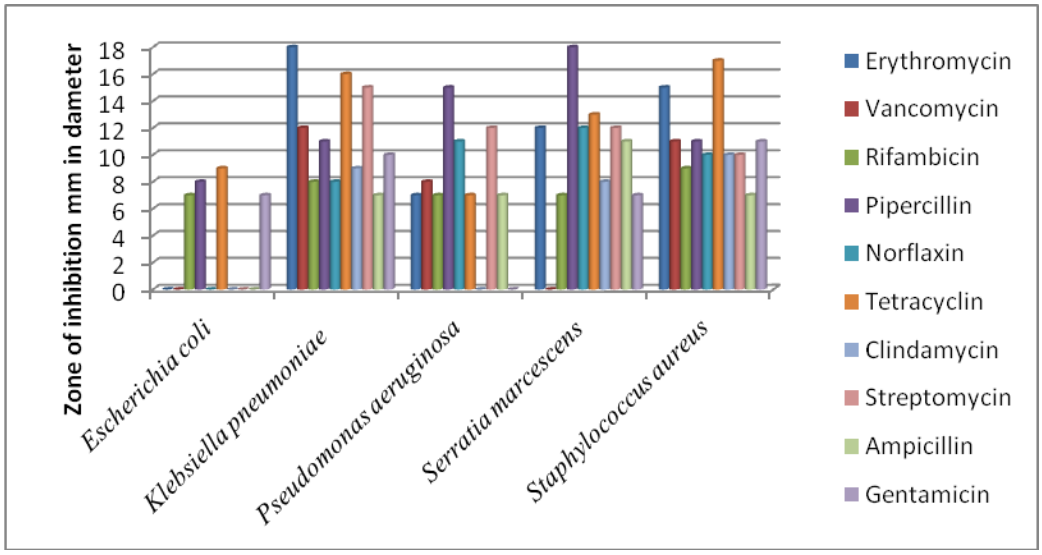

Fig. 2: Antibacterial Activity

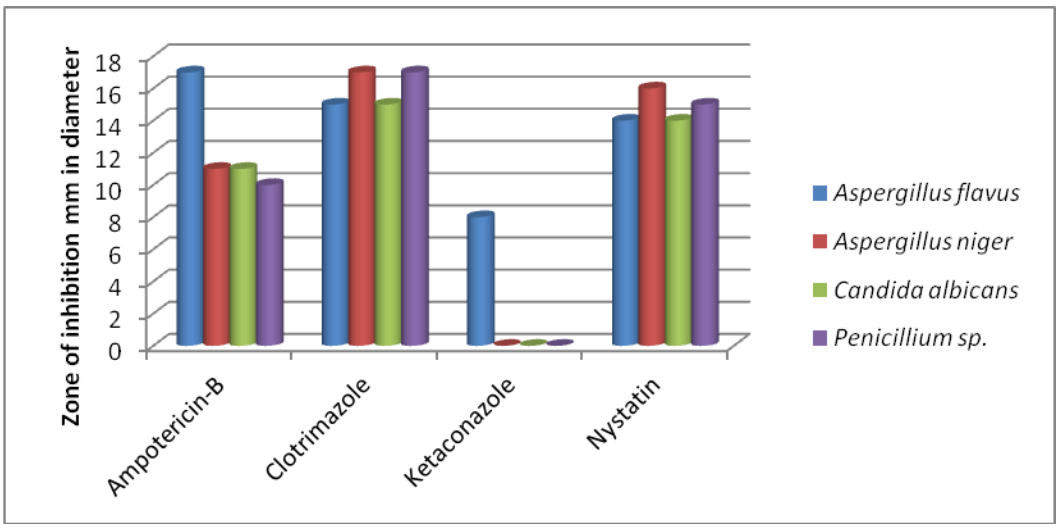

Fig. 3: Antifungal Activity

CONCLUSION

Antimicrobials with anticipated effectiveness in kitchen environment pathogenic insufficiency are selected Pipeareillin and Tetracycline. Clindamycin should not be used because of low antibacterial activity against all isolated bacteria. Similarly, clotrimazole drug was most effective in isolated pathogenic fungi.

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