Isolation of *Klebsiella pneumoniae* and *Acinetobacter radioresistens* from Dish wash Scrubbers

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ABSTRACT

Aims: To identify predominant microorganisms in dish washing scrubbers collected from ten different sources.
Study design: Collection of dish wash scrubbers from different sources, pour plating of appropriately prepared dish wash scrub suspensions and subsequent isolation and identification of predominant isolates. Assessment of antibiotic susceptibility of the selected isolates by disc diffusion assay.
Place and Duration of Study: April, 2018 - June 2018

Methodology: A total of 10 dish wash scrubbers (synthetic green scrubber pads free from any anti-bacterial preservatives belonging to the same brand) were collected from various sources. Appropriately prepared dish wash scrub suspensions in peptone water were pour plated on Plate Count Agar (PCA) and MacConkey agar. Predominant colonies selected from the plates based on the colony morphology were subjected to Grams staining, catalase, oxidase, indole, citrate, urease tests and genotypic identification by 16S ribosomal RNA sequencing. The identified isolates were tested for their susceptibility to eight antibiotics by disc diffusion method.
Results: Irrespective of the sample source, most of the dish wash scrubbers sampled harbored similar types of colonies. From the colonies obtained two of them were identified by 16S rRNA sequencing and subsequent blasting as *Klebsiella pneumoniae* and *Acinetobacter radioresistens*. The isolates were deposited in the NCBI database with accession numbers *MK032217 (Klebsiella pneumoniae* RSV02) and *MK032134* (*Acinetobacter radioresistens* RSV 01). These isolates were tested for their susceptibility to different antibiotics and *Acinetobacter radioresistens* RSV 01 was found to be more antibiotic susceptible than *Klebsiella pneumoniae* RSV02.
Conclusion: Observations of this study confirm the potential role of dish wash scrubbers as vehicle for potential pathogens and their ability to act as cross contaminating agents in food processing environments.

Keywords :Enterobacteriacea , Moraxellaceae, Antibiotic susceptibility

1. INTRODUCTION

Food contact surfaces can present a potential health hazard if they are not properly cleaned and sanitized. Contaminated surfaces of utensils, sponges, cutting boards and equipment have been identified as sources of cross-contamination for food during preparation and service to consumers. Among these, kitchen sponges/ dish wash scrubbers offer ideal places for harmful bacteria and other pathogens, such as viruses, to grow [1]. With the wide availability of dish wash scrub pads/ kitchen sponges in Indian market they have become the material of choice to the extent that they have tremendously replaced the traditionally used options like coconut husk and mature fruit of *Luffa acutangula* (peechinga) for removing food residues and other debris from kitchen vessels and utensils. Because of the wet environments and ready availability of nutrients owing to repeated contact with food, sponges/scrubbers can serve as potential growth environment for bacteria. The moist, micro-crevices that make a scrubber an effective cleaning device also make it a congenial

home for germs, which is very difficult to disinfect. So these dish wash scrubbers could present a potential health hazard If not properly cleaned and sanitized as these dish wash sponges are frequently used not only to clean dishes but also to clean various surfaces increasing the risk of cross-contamination. Presence of pathogenic organisms like *Campylobacter* spp., *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Salmonella* spp. and *Staphylococcus* spp. are reported in kitchen scrubbers [2]. Existence of pathogenic organisms in kitchen sponges is an area of serious food safety concern considering the cross contamination challenges they pose. Many studies have correlated the occurrences of food borne illness outbreaks to cross-contamination from raw products via hands, cleaning cloths or sponges used especially in the case of foods that were not subjected to further cooking. This study was conducted to isolate and identify the predominant bacteria present in dish wash scrub pads.

2. MATERIAL AND METHODS

2.1 Isolation and Identification of predominant isolates

A total of 10 dish wash scrubbers (synthetic green scrubber pads free from any anti-bacterial preservatives belonging to the same brand) were collected aseptically in a sterile container. The samples were collected on an average after two weeks of its use and were analyzed in Department of Dairy Microbiology, College of Dairy Science and Technology , Mannuthy, Thrissur [3]. For this, from the collected kitchen sponge samples, 25 mm^3 was aseptically cut using sterile blade and the pieces were blended in 250 mL of sterile peptone water. After thorough mixing, appropriate dilutions were prepared using peptone water and pour plated in Plate Count Agar (PCA) and MacConkey agar and incubated at 37^0C for 48 h and 24 h respectively. From each of the growth medium and sample, predominant colonies were selected based on their colony morphology and stored at 4 □C till further procedures. Out of these isolates, two most frequently encountered colony types were subjected to Gram staining, catalase, oxidase, citrate, indole and urease tests. Genotypic identification of these isolates was carried out by 16S ribosomal RNA (16Sr RNA) sequencing at Rajiv Gandhi Center for Biotechnology, Trivandrum. 16S-RS-F (CAGGCCTAACACATGCAAGTC) and 16S-RS-R (GGGCGGWGTGTACAAGGC) were used as forward and reverse primers respectively. The sequences obtained were searched with the NCBI BLAST program (http://www.ncbi.nlm.nih.gov) for their closest relative/reference strains with a homology of over or equal to 99 per cent. The sequences were deposited in NCBI Genbank using BankIt (https://www.ncbi.nlm.nih.gov/WebSub/) program

2.2 Assessment of antibiotic susceptibility of the isolates

The identified isolates were tested for their sensitivity to eight antibiotics namely Amoxycillin(10µg), Ampicillin(10µg), Clindamycin(10µg), Gentamycin (120µg), Ofloxacin(5µg) Penicillin(10µg), Streptomycin(10µg) and Tetracycline(30µg) by the disc diffusion method. For this, active cultures of the isolates adjusted to an O.D. of 0.5 McFarland standard was swabbed over the surface of pre prepared Mueller Hinton agar (Himedia, Mumbai) Petri dishes and the antibiotic discs were placed over it [4]. The plates were incubated at 37 □C for 24 hours and the diameters of zones of clearances were measured and recorded.

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of predominant isolates

Out of the colonies developed two repeatedly appeared colony types were considered as the most predominant ones and subjected to phenotypical characterization. Both the isolates were found to be catalase positive, oxidase and indole negative (Table 1). Isolate 1 gave

positive citrate and urease tests whereas isolate 2 gave negative results for these tests. By sequencing their 16S rRNA amplified PCR sequences and comparing them with the GenBank database using the BLAST program the first isolate was identified as *Klebsiella pneumoniae* and the second isolate was identified as *Acinetobacter radioresistens*. The isolates were deposited as *Klebsiella pneumoniae* RSV02 and *Acinetobacter radioresistens* RSV 01 with accession numbers MK032217 and MK032134 respectively.

Table 1.Phenotypic Characteristics of the isolates

Isolate No.	Growth Media used	Gram staining	Catalase	Oxidase	Indole	Citrate	Urease
1	MacConkey agar	Short, gram negative rods arranged singly and in pairs as bundles	+	-	-	+	+
2	Plate Count Agar	Short, gram negative coccobacilli arranged singly or in clusters	+	-	-	-	-

+ - Positive reaction, - Negative reaction

Isolation of *Klebsiella pneumoniae* a pathogenic member of the family *Enterobacteriacea* from kitchen sponges is of serious concern and had been reported in previous studies also [5,6, 7]. The situation is worrying, considering that *Klebsiella* is one of the most widespread opportunistic pathogens with fatal prognosis [8]. Isolation of *Acinetobacter radioresistens* belonging to the family *Moraxellaceae* in the current study is very much in agreement with the previous reports [2,5, 9]. Findings of the current study agrees with the suggestion of these researchers that bacteria affiliated with the *Moraxellaceae* seem “typical” for kitchen sponges. Contamination of kitchen sponges with potentially pathogenic bacteria like *Bacillus* and *Staphylococcus* was reported in a recent study also [10].

3.2 Assessment of antibiotic susceptibility of the isolates

The isolate *Klebsiella pneumoniae* RSV 02 was found to be not affected by six out of the eight antibiotics tested whereas *Acinetobacter radioresistens* RSV 02 was found to be not affected by two of the antibiotics namely Ampicillin and Tetracycline (Table 2). It is an

interesting observation considering the well established resistance of *Acinetobacter radioresistens* strains to radiations and various antibiotics.

Table 2. Antibiotic susceptibility of the isolates

Sl. No	Name of antibiotic	Diameter of zone of inhibition (mm)	
		Klebsiella pneumoniae RSV 02	*Acinetobacter radioresistens* RSV 01
1	Amoxycillin	0	22
2	Ampicillin	0	0
3	Clindamycin	0	23
4	Gentamycin	0	24
5	Ofloxacin	0	26
6	Penicillin	0	30
7	Streptomycin	11	18
8	Tetracycline	24	0

4. CONCLUSION

This study isolated *Klebsiella pneumoniae* and *Acinetobacter radioresistens* from dish wash scrubbers. Isolation of potentially pathogenic organisms like *Klebsiella pneumoniae* from dish was scrubbers is of serious concern and alerts us about the possibility of scrubbers being a potential source of contamination in food processing environment. Outcome of this study emphasis the relevance of making the general public aware of the risks associated with the use of dish wash scrubbers, the necessity of frequent change of kitchen sponges and adoption of periodic efficient decontamination treatments.

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