

# ISOLATION AND IDENTIFICATION OF BACTERIAL CONTAMINATION FROM COMMONLY USED HOUSEHOLD SURFACES

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## ABSTRACT

**AIM:** Microbial populations in indoor environments, where we live and eat, are important for public health. So the purpose of this study is to isolate and identify the bacterial contamination from common household. **METHODS:** Five samples of bacteria were collected from pillow cover, bed sheets, bathroom floor, laptop, and wash basin. Each sample was cultured on the nutrient agar plates and cultural characterization was done on the basis of colony morphology and gram staining. Each sample was further characterized on the basis of standard biochemical tests and its antibiotic sensitivity was tested against antibiotics (Norfloxacin, Azithromycin, Sulphamethoxazole, Cefixime, Tetracycline, Penicillin, Ciprofloxacin, Oxacillin and Cefuroxime) using disc diffusion method. **RESULTS:** Gram-negative and gram positive bacilli which appeared rod-shaped pinkish and purple colored colonies appeared under the microscope. Five isolates were characterized on the basis of biochemical tests that showed Gram-positive (*S.aureus* and *S.pneumoniae*) and Gram-negative (*Shigella*, *E.coli* and *K.pneumoniae*) bacteria in the isolated samples from household surfaces. The results of microbiological tests of household goods can be used to emphasize the importance of the sanitary conditions in the house.

**Keywords:** Antibiotics, disc diffusion method, gram staining

## INTRODUCTION

Microorganisms are ubiquitous in nature. The microbial-mediated contamination of food, air, water, and surfaces of common households may cause specific site-borne illnesses. Unless the cause of infection is determined due to microbial toxins (e.g. *Shigella*, *Salmonella*, *Campylobacter*, *Listeria*, *Clostridium*) (Jarvis *et al.*, 2012, Arias and Murray, 2012) the microbial contamination in humans is usually symptom-free (Miyajima *et al.*, 2011). The bacteria *Salmonella*, *Shigella* and *Escherichia coli* are among the common microorganisms that cause fatal illnesses in humans (Bush, 1989, Hoppe, 1993). Microbial contamination of indoor has been studied in the context of human health using culture-dependent and -independent techniques. Most studies focused on the bacterial contamination of surfaces in kitchens and restrooms, which are readily colonized by microbes (Flores *et al.*, 2011; Flores *et al.*, 2013; Kembel *et al.*, 2012; Ojima *et al.*, 2002, Rintala *et al.*, 2008). It is very easy to find locations in a house that are inadequately cleaned because these locations are used routinely. For example kitchen counters, kitchen sinks, bathroom floors, toilet seats, and common household items may not be adequately cleaned and hence can carry microorganisms that can cause life-threatening diseases (Beutin and Martin, 2012, Soares and Ahmer, 2011, CDC, 2012). Many household locations are always susceptible to microbial exposure, particularly in family dwellings where many facilities are shared. Identification of the sources of bacterial contamination in indoor environment is important for managing food safety. Human skin is a primary source of bacteria in indoor environments, and individuals can transmit bacterial pathogens by touching indoor spaces (Flores *et al.*, 2011, Flores *et al.*, 2013). The alcohol-based sanitizer has been recommended as an effective tool to sanitize various surfaces, including hands (Burton *et al.*, 2011, WHO, 2012, Pickering *et al.*, 2011).

Recently, a German outbreak caused by Shiga-toxin producing *Escherichia coli* O104:H4 showed that unwashed vegetables could be a risk element (Buchholz *et al.*, 2011). Mostly previously reported culture-dependent studies of a kitchen and refrigerator microbes focused on pathogen detection (Evans *et al.*, 2004, Jackson *et al.*, 2007, Ojima *et al.*, 2002, Ojima *et al.*, 2002, Sinclair and Gerba, 2011). The recent advent of next generation sequencing techniques provides unprecedented data on the microbial composition, and the ecology of various environments, including indoor spaces (Flores *et al.*, 2011; Flores *et al.*, 2013).

It is well known that food items contains pathogenic bacteria such as *Campylobacter*, *Salmonella*, and *Listeria* (Heaton and Jones, 2008; Luber, 2009; Berger *et al.*, 2010), and that proper kitchen hygiene is critical for minimizing the spread of such disease-causing organisms (Rusin *et al.*, 1998; Cogan *et al.*, 1999; Scott, 2000; Cogan *et al.*, 2002). However, the full extent of bacterial diversity remains largely unknown in kitchens as most of the earlier studies of the microbial community in the kitchen focused on pathogen detection and relied upon cultivation-dependent techniques that preclude in-depth community characterization (Scott *et al.*, 1982; Ojima *et al.*, 2002a; Ojima *et al.*, 2002b; Sinclair and Gerba, 2011). Nevertheless, from these studies, it is apparent that both gram-negative and gram-positive bacteria can readily be cultivated from a variety of kitchen surfaces, with moist surfaces typically yielding the greatest number of colony-forming units. One of the bacterial disease with the highest burden is tuberculosis, caused by the *Mycobacterium tuberculosis*, which kills about 2 million people, about a year, mostly in sub-Saharan Africa. Many types of pathogenic bacteria contribute to another globally important disease, such as pneumonia caused by *Streptococcus* and *Pseudomonas*, many types of bacteria are responsible for foodborne diseases which can be caused by *Shigella* and *Campylobacter* bacteria. Some pathogenic bacteria are responsible for infections such as tetanus, typhoid fever, diphtheria, syphilis and leprosy. Pathogenic bacteria are also the cause of high infant mortality rates in many developing countries. Many types of pathogenic bacteria are responsible for human death in non-curable conditions, because the infection spread rapidly in some severe cases, if the patient suffers the other type of diseases and with poor immune system.

## MATERIAL AND METHODS

### Collection of samples

Different samples were collected from different common household surfaces. Surfaces like pillow cover, bed sheets, bathroom floor, laptop, wash basin were taken (Table 1). Samples were inoculated in nutrient culture media in five different nutrient broth tubes and incubated for 24 hrs at 37°C. After incubation, each different petri plate was poured and spread over the plates. After spreading, the plates were incubated at 37°C for 24 hrs.

Table no-1: Isolation of isolates

Sample no.	Location of collections
1	Pillow cover
2	Bed sheets
3	Bathroom floor
4	Laptop

5	Wash basin
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**Antimicrobial agents:** The following antibiotic discs at the final concentrations that are indicated were used: Norfloxacin (10 mcg/disc), Ciprofloxacin 5 mcg/disc, Oxacillin (1 mcg/disc), Azithromycin (10 mcg/disc), tetracycline (30 mcg/disc), cefixime (5mcg/disc), Penicillin (10 unit/disc), Cefuroxime (30 mcg/disc) and Sulfamethoxazole (25 mcg/disc). All the antibiotic discs were obtained from Himedia except norfloxacin which was obtained from Sisco Research Laboratories Pvt. Ltd.

### Preparation of Media

The dehydrated culture medium, nutrient agar (NA), obtained from Thermoscientific was prepared. NA medium was prepared and sterilized by autoclaving at 121°C for 15 minutes. The medium was cooled to 60-65°C and poured into sterile disposable petri dishes (95×15mm). A medium was solidified at room temperature and incubated at 37°C overnight to check for possible contamination. The plates were stored at 4°C in an airtight polythene bag until they were used for the isolation of microorganisms.

### Isolation of microorganisms from house locations

The microorganisms were examined from routinely used surfaces of different locations in a house (i.e. wash basin, laptop, bathroom floor, pillow cover and bedsheets). The cotton swabs were sterilized by autoclaving at 121°C for 15 minutes, and stored at room temperature until ready to be used. The sterile cotton swabs were swabbed at one-inch sections of wash basin, laptop, bathroom floor, pillow cover and bedsheets and swabbed individually on separate sterile NA plates. After drying the surface, the surfaces were swabbed with sterile cotton swab on NA plates. After streaking, the plates were incubated at 37°C for 24 hours. The microbial growth was observed after incubation.

**Identification and characterization of isolated strains:** The isolates of bacteria were identified on the basis of standard microbiological methods:

**Cultural characteristics:** on the basis of morphology of the colony the samples were characterized which included following properties: appearance, color, shape of the colony, margin, elevation and gram staining. Gram stain was the key step and was carried out according to Harrigan and McCance (1966).

**Biochemical characterization:** Each sample was further characterized on the basis of their biochemical test such as indole, catalase, oxidase, carbohydrate fermentation, H<sub>2</sub>S, urease test to identify the organism to the species level, using Bergey's manual of determinative bacteriology, (1939).

### Antimicrobial Susceptibility Testing

**Procedure:** Antibiotic discs were used for antibiotic sensitivity test by using disc diffusion method (Ehinmidu, 2003). Discs were applied to the bacterial agar plate using sterile needles against each sample. After measuring distance 4-5mm between each disc, plates were incubated at 37°C for 24 hrs. After incubation period, a clear zone formed around antibacterial discs and the diameter of zone of inhibition was measured using a millimeter ruler. The results were taken according to NCCLS (1979).

# RESULT AND DISCUSSION

## Characterization of recovered isolates

Characterization of isolates was performed by culturing on Nutrient Agar, Gram's staining and biochemical tests.

## Culture of recovered isolates

Five clinical isolates were procured from different sites. All of five samples were cultured on nutrient agar.

## Gram's staining

Gram-negative and gram positive bacilli which appeared rod-shaped pinkish and purple colored colonies appeared under the microscope.

**Biochemical Characterization:** Five isolates were characterized on the basis of biochemical tests. The tests performed to characterize the isolates were Indole, MR, VP, citrate utilization, nitrate reduction, lactose, sucrose and glucose fermentation (**Table 2**). Catalase was positive for all sample except sample 3 and urease was positive for sample 1 while other four samples were negative for urease. Methyl red showed positive test except in sample 5 whereas Voges Proskauer test was positive for only sample 1 and sample 5. Oxidase and H<sub>2</sub>S tests were negative for all isolated samples, whereas fermentation was positive for all the isolated samples.

**Table no-2: Identification of organisms by biochemical tests in collected samples**

Sample No.	Methyl Red	Voges Proskauer	Indole (I)	Catalase (C)	Oxidase	H <sub>2</sub> S	Urease	Fermentation		
								G	L	S
1	+	+	-	+	-	-	+	+	+	
2	+	-	+	+	-	-	-	+	+	
3	+	-	+	-	-	-	-	+	+	
4	+	-	+	+	-	-	-	+	+	
5	-	+	-	+	-	-	-	+	+	

(+)= Positive; (-)= Negative; G=Glucose; L= Lactose; S= Sucrose

Biochemical characterization and gram staining of samples showed different recovered isolates in each sample as shown in **table 3**.

**Table 3: Recovered isolates on the basis of gram staining and biochemical tests**

S.No.	Gram staining	Recovered isolate
1	Gram positive	<i>S. aureus</i>
2	Gram negative	<i>Shigella</i>
3	Gram positive	<i>S. pneumoniae</i>
4	Gram negative	<i>E.coli</i>
5	Gram negative	<i>K. pneumoniae</i>

**Antibiotic sensitivity test was done by the disc diffusion method.**

The placing of a antibiotic discs (measuring 6mm diameter) is an known amount of an antibacterial agent on the agar surface preciously inoculated with isolates to be tested that resulted in zone of inhibition of bacterial growth around the disc. Results of recovered isolates and measurement of zone of inhibition is given below in the **table no.4**

**Table no.4: Antibiotic susceptibility test of bacterial isolates obtained from household samples**

Sample No	Nfx	AZM	S	CFM	TET	P	CIP	OX	Cf
<b>Zone of Inhibition (mm)</b>									
1	10.2	25.2	20.5	-	17.4	22.4	26.3	10.2	22.3
2	20.5	20.6	25.1	-	18.2	-	25.9	-	20.6
3	-	30.3	16.8	15.4	20.9	20.2	24.8	-	24.3
4	-	20.5	26.3	-	19.6	17.4	23.5	-	20.7
5	-	--	15.8	17.4	15.6	-	25.3	-	25.2

(-)= No Zone of inhibition; Nfx = Norfloxacin; AZM = Azithromycin; S = Sulphamethoxazole; CFM = Cefixime; TET = Tetracycline; P = Penicillin; CIP = Ciprofloxacin; OX = Oxacillin; Cf= Cefuroxime

# DISCUSSION AND SUMMARY

It is interesting to demonstrate the ubiquity of microbes in a home environment, which we considered to be a safe place, devoid of microbes. A motivation for this study was the numerous reports about the occurrence of pathogenic microorganisms on household surfaces. Our study revealed varied microbial loadings from routinely used sites. In the present study, the appearance of microorganisms was observed on nutrient agar plates which were swabbed with samples from five different sites of a house (wash basin, laptop, bathroom floor, pillow cover and bedsheet) under non-sterile conditions. The study of the microbial complexity from routinely used sites within the household surfaces was based on the color, size, and appearance of microorganisms. However, the identification of commonly found microorganisms from household surfaces enable us to determine the conditions under which most microbial outbreaks may occur within households.

In our study, five isolates were characterized on the basis of biochemical tests that showed Gram positive (*S. aureus* and *S. pneumoniae*) and Gram negative (*Shigella spp.*, *E. coli* and *K. pneumoniae*) bacteria in the isolated samples from wash basin, laptop, bathroom floor, pillow cover and bedsheet. Similarly, in one of the previous study of Othman, 2015 reported that the five (84%) kitchens were found to harbor pathogenic microorganisms such as *E. coli*, *Klebsiella spp.*, *S. aureus*, *S. epidermidis*, *Salmonella spp.*, *Shigella spp.*, and *Micrococcus spp.* A similar study (Adiga *et al.*, 2012) was conducted, which found that 64% of sample collected were contaminated with pathogenic microorganisms such as *Klebsiella pneumoniae*, *Proteus spp.*, *S. epidermidis*, *E. coli*, *S. aureus*, and *Enterobacter spp.* He also found that *K. pneumoniae* was the most abundant and prevalent bacteria in the kitchens. This result was in agreement with ours, as *Klebsiella spp.* and *E. coli* is present in the tested kitchen sites and concluded that *E. coli* and *Klebsiella spp.* were the most abundant bacteria which reveals the poor hygiene in kitchen. These studies support the findings of the present study. Even though these locations are routinely cleaned, the presence of microorganisms is assumed to be a part of the microbial flora. The large numbers of microbial flora or NA plates from uncleaned surfaces suggest that we should use alternative and non-hazardous materials to sanitize our households daily.

The microbial flora observed from the un-cleaned surfaces of various locations in the house can be considered as non-pathogenic microbiota. Non-pathogenic microorganisms can mutate into a virulent phase, liberating toxins and causing illness (Brussow *et al.*, 2004). In general, the normal human microbiota can protect the human body from several microbial types (Pillinger and Blaser, 2007). Consequently, microbial pathogenesis are currently a major concern within households, where most sources of microbial infection remain unnoticeable.

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