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Currency notes in circulation can be potential sources of transmissible diseases

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Abstract:

In the modern era, currency is the primary medium for exchange of goods and services. The material of Indian currency notes makes it a hub for microorganism to thrive. Taxi drivers exchange currency with customers for different regions and backgrounds and meat sellers are known to use bare hands for butchering of the meat and exchanging currency with customers without washing or sanitizing. This paper highlights the presence of multitude of bacteria which have the potential to cause diseases as well as being antibiotic resistant. 20 different strains were isolated, 10 each from currency notes obtained from a local taxi driver and meat seller. Biochemical tests for identification of these bacteria were performed which suggests presence of bacteria such as *Vibrio gazogenes, Chrysomonas luteola, Vibrio cincinnatienses, Vibrio metschnikovii, Staphylococcus intermedius, Vibrio gazogenes, Flavimonas oryzihabitans, Vibrio gazogenes, Micrococcus mucilaginous, Salomonella sp., Micrococcus luteus, Staphylococcus simulans, E. coli, Photobacterium angustum, Streptococcus sp., Pseudomonas mallei, Salmonella paratyphi and Pseudomonas malle.* The isolated bacteria were resistant to Penicillin, Ceftizoxime and Gentamycin with some Gram-positive bacterium being resistant to vancomycin, ceftizoxime and penicillin with gentamycin showing little to no effect.

Keywords: Bacteria, Antibiotics, Pathogen, Currency notes, circulation

1.1 Introduction:

Currency is the primary medium of exchange in the modern world, having long ago replaced bartering as a means of trading goods and services (Felgo and Nkansah, 2010). The Indian rupee's rich history traces back to circa 6th century BCE of ancient India, and was one of the earliest issuers of coins in the world (Kapoor, 2002*)*. But there is a huge possibility of microbial contamination and spread of diseases through currency exchange (Prasai *et al.,* 2009). Internationally, paper currency is made of a rugged mix of 75%

cotton and 25% linen, and offers surface area for bacteria and microorganisms to reside on both sides (El-Dars *et al.,* 2005). According to the Reserve Bank of India (RBI), an Indian note uses 100 percent cotton. Cotton-based currency notes contained three times higher bacterial counts than polymer-based ones concluding that the spaces between the cotton fibers provide favourable anchor for the bacteria (Vriesekoop *et al.,* 2010*).*

There are many modes of microbial transmission like air, water, physical contact, food etc. (Pradeep *et al.,* 2012). Pathogen may spread through touching of currency notes. Due to lack of hand washing/sanitizing, there is possibility of spread and ingestion of pathogen as it is local practice to eat with bare hands, along with the practice of licking the tip of the finger while counting money and making skin contact.

Being a popular tourism state, and home to many prestigious educational institutions, where students from all over India study and reside, the local taxi drivers of Meghalaya exchange currency with local customers as well as tourists/students from all over India and abroad which may lead to inhabitation of a wide range of flora.

Local meat sellers have been observed to use bare hands to touch, cut meat and exchange currency with customers without hand washing or sanitizing. A variety of microbes can contaminate meat, depending on pH, oxygen, water availability, and storage temperatures different species may become dominant (Ercolini *et al.,* 2006). Foodborne pathogens such as *Salmonella* sp. and *Escherichia coli* contaminate the carcass and spread to the raw meat (Lecos *et al.,* 1987*).* The currency notes may become contaminated via touch and later exchanged with unsuspecting customers.

Emergence of antibiotic resistant bacteria has led to a health crisis in recent years, especially in developing countries where relatively easy availability and higher consumption of medicines have led to disproportionately higher incidence of antibiotics resistance compared to developed countries (Geneva, 1996). According to the World Health Organization, the epidemiological aspects of antimicrobial resistance in most of South East Asian countries is not well known. Antimicrobial resistance will result in difficulty in controlling the diseases in the community and ineffective delivery of the health care services. Meta analyses of the drug susceptibility results of various laboratories in India reveal an increasing trend of development of resistance to commonly used antimicrobials in pathogens like *Salmonella*sp.*, Shigella*sp.*, Vibrio cholerae, Staphylococcus aureus, Neisseria gonorrhoeae, N. meningitidis, Klebsiella*sp., *Mycobacterium tuberculosis*, HIV, plasmodium and others (Felgo and Nkansah, 2010).

The study considered currency notes of ten rupees randomly from a taxi driver and twenty rupees notes from a meat seller. In this study,the microfloraof bacteria existing in

these notes were examined for presence of antibiotic resistant strains and established a comparison with drinking water to raise awareness keeping in mind thepandemic situation which is connected with the spread of microorganisms from everyday material.

1.2 Study area

East Khasi Hills is an administrative district in the state of Meghalaya in India. The district headquarter is located at Shillong. The district occupies an area of 2752 km² and has a population of 825,922 (as of 2011). East Khasi Hills District forms a central part of Meghalaya and covers a total geographical area of 2,748 km2. It lies approximately between 25°07" & 25°41" N Latitude And 91°21" & 92°09" E Longitude. The present study considered the isolation and characterization of bacteria from currency notes collected from a local taxi driver and meat seller of the East Khasi Hills.

1.3 Methodology

1.3.1 Sampling

Samples were collected from a local taxi driver designated as Sample 1 and meat seller designated as Sample 2 using sterile disposable gloves and autoclaved sampling bottles. Currency notes of denominations of Rs 10 and Rs 20 were randomly collected from a local taxi driver and meat seller respectively. To ensure proper touch by meat seller, chicken meat was bought and currency was exchanged.

Samples were transferred to two sterile flasks and standard method was followed to get the microbes on the solution as the source of microbial samples.

1.3.2 Isolation of bacteria

Serial dilution of the samples was done using sterile test tubes which were labelled 10^{-1} to 10^{-7}dilutions. 9 ml of 0.85% NaCl were then measured into the seven test tubes. 1 ml of sample solution was introduced into the first test tube labelled 10^{-1} and mixed thoroughly, and 1 ml was taken from the first test tube and transferred to the second test tube labelled 10^{-2}. This was continued until the 10^{-7} dilution was obtained. 0.1ml of samples from 10^{-5}, 10^{-6} and 10^{-7} dilutions were inoculated on each nutrient agar plates by the spread plate technique. The plates were then incubated at 37°C for 18-24 h. The growing colonies on the plates were recorded as colony forming unit (CFU) (Brown,2009) and the number of bacteria in the samples was expressed as CFU/ml which was calculated as CFU X Dilution factor/ Volume of sample plated.

1.3.3 Pure culture generation

Based on the colony morphology, 20 of the most prominent bacterial colonies, 10 from each sample were isolated using sterile loop and streaked into nutrient agar plates. Resultant single distinct colonies were sub-cultured in slants and maintained at 4°C for further studies.

1.3.4 Differential staining using Gram's stain

Gram's staining technique was used to find out the Gram staining nature of the bacterial isolates. A loopful of fresh overnight grown bacterial culture was smeared on a clean slide and then allowed to air dry. The smear was then heat fixed by passing the glass slide over a flame. Few drops of primary stain i.e., crystal violet was added and left for 60seconds and then washed with distilled water. Gram's iodine, a mordant was added for 1 minute and the slide was washed away with Gram's decolourizer (ethyl alcohol 95%). Finally, safranin, counter stain was added for 1 minute which was then washed with distilled water. The glass slide was left to air dry and examined under the microscope.

1.3.5 Biochemical characterization

1.3.5.1 Catalase test: This is a test to ascertain the ability of bacteria to produce catalase that reduces hydrogen peroxide to water and oxygen. Growth of samples were scraped with wired loop. It was then suspended in a drop of 3% H_2O_2 on a slide, then examined for bubble formation. If effervescence occurs, it is confirmatory positive test for catalase production, but if it does not occur it is negative test for catalase production.

1.3.5.2 Oxidase test:

This test depends on presence of certain oxidases in bacteria that will catalyse the transport of electrons between electron donors in the bacteria and a redox dye- tetraethyl p-phenylene-diamine which is reduced to a deep purple if positive. A strip of filter paper was moistened with freshly prepared 1% solution of the reagent. Immediately, a speck of culture was rubbed on it with a loop. Positive test is indicated with an intense deep purple blue within 10-60 seconds. No colour change after 60 seconds indicates a negative result.

1.3.5.3 Citrate Utilization test:

Citrate is acted upon by enzyme citrase which produces oxaloacetic acid and acetate. These are enzymatically converted to pyruvate and CO_2. During reaction, the medium becomes alkaline as the CO_2 combines with Na and H_2O to form sodium carbonate which is alkaline. Simmon's citrate medium slants were prepared. Samples were inoculated into

agar slants and incubated for 24-48 hrs at 37°. Positive result is indicated by blue colour slope and no colour change indicates a negative result.

1.3.5.4 Triple Sugar Iron (TSI) test:

This test depends on ability of bacteria to ferment lactose, sucrose and glucose and the production of hydrogen sulphide. TSI agar medium was prepared, dispensed in test tubes, sterilized and allowed to set as slopes. Slants were inoculated with samples and incubated for 18-24 hours at 37°C. Yellow butt, red slant indicates positive glucose fermentation. Yellow butt, yellow slant indicates positive lactose and/or sucrose fermenting. Red butt, red slant indicates neither glucose, lactose, sucrose fermenting. Black precipitate at bottom of slant indicates H_2S production.

1.3.5.5 Methyl red test:

This test detects ability of microbes to oxidise glucose with production and stabilization of high concentration of acid end products. MR-VP broth was prepared, sterilized and inoculated with samples and incubated for 48 hours at 37°C. Following incubation, 5-6 drops of methyl red solution was added. Bright red colour change indicates positive result, red- orange colour indicates a weak positive result and yellow- orange indicates a negative result.

1.3.5.6 Voges-Proskauer test:

The Voges-Proskauer (VP) test is used to determine if an organism produces acetyl methyl carbinol from glucose fermentation. If present, acetyl methyl carbinol is converted to diacetyl in the presence of ∝- naphthol, strong alkali (40% KOH), and atmospheric oxygen. MR-VP broth was prepared and inoculated with samples and incubated for 48 hours at 37 °C. Following inoculation, Barritt's reagent A and Barritt's reagent B was added to the broth in a 3:1 ratio. Tubes were shaken at intervals to ensure maximum aeration. A positive result is indicated by the development of a pink colour in 2.5 minutes, becoming crimson in 30 seconds and no colour change indicates a negative result

1.3.6 Antibiotic susceptibility by Kirby's disk diffusion assay

This method relies on the inhibition of bacterial growth measured under standard conditions. For this test, Mueller-Hinton agar was uniformly and aseptically inoculated with the test bacterial samples. Antibiotic discs were placed on the surface of the agar aseptically. The plates were then incubated for 24 hours at 37°C.

The organism grows on the agar plate while the antibiotic inhibits the growth. If the organism is susceptible to a specific antibiotic, there will be no growth around the disc

containing the antibiotic. Thus, a "zone of inhibition" can be observed and measured to determine the susceptibility to an antibiotic for that particular organism. The measurement is compared to the criteria set by the Clinical and Laboratory Standards Institute (CLSI). Based on the criteria, the organism can be classified as being Resistant (R), Intermediate (I) or Susceptible (S)

1.4 Observation and results

1.4.1 CFU calculation:

Serial dilutions of the samples at 10^{-1}, 10^{-2},10^{-3}, 10^{-4},10^{-5}, 10^{-6} and 10^{-7} dilutions were prepared and dilutions of 10^{-5}, 10^{-6} and 10^{-7} were plated. Countable colonies were observed only in the 10^{-7} plate and considered for bacterial enumeration.

It is to be noted that samples of soil and fresh water which are hubs for bacterial flora usually provide countable colonies in 10^{-3} or 10^{-4} dilutions. However too numnerous to count (TNTC) w convey the higher bacteria carrying capacity of the samples. The dilution plates and the streak plates are presented in the Fig 1a and Fig 1b respectively. The summary of the CFU obtained is shown in Table 1.

A comparison was established with drinking water as control and 0.1 ml of the sample was plated along with 10^{-1} and 10^{-2} dilutions where no growth was observed.

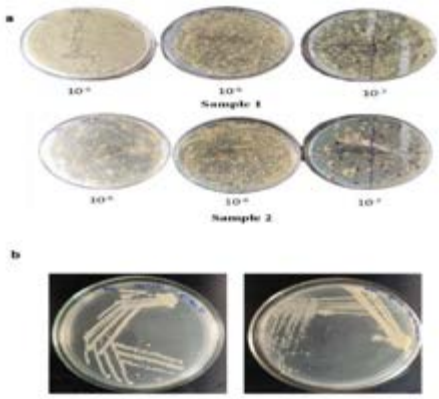

Fig 1: (a) CFU of sample 1 and sample 2 (b) Streak plates

Table 1. Summary of CFU obtained for Sample 1 and Sample 2

Sample 1		**Sample 2**	
Dilution	No. of colonies	Dilution	No. of colonies
10^{-5}	TNTC	10^{-5}	TNTC
10^{-6}	TNTC	10^{-6}	TNTC
10^{-7}	290	10^{-7}	230
Sample		CFU/ml	
Sample 1		2.9×10^{10}	
Sample 2		2.3×10^{10}	

(TNTC- Too numerous to count)

1.4.2 Gram staining

In this study, out of the total 20 isolates obtained from Sample 1, 2 isolates were found to be Gram positive and 8 isolates were found to be Gram negative. Similarly, from Sample 2, 3 isolates were found to be Gram positive and 7 isolates were found to be Gram negative. The colony morphology and the Gram nature of the isolates are shown in Fig 2 and Table 2.

Table 2. Colony morphology and Gram nature of the isolates obtained from Sample 1 (M1-M10) and sample 2 (T1-T10)

Isolates	Gram positive	Gram negative	Morphology
M1	-	+	Cocci
M2	-	+	Bacilli
M3	-	+	Bacilli
M4	-	+	Cocci
M5	+	-	Cocci
M6	-	+	Cocci
M7	-	+	Cocci
M8	-	+	Bacilli
M9	+	-	Chain
M10	-	+	Bacilli
T1	+	-	Cocci
T2	+	-	Cocci
T3	-	+	Bacilli
T4	-	+	Cocci
T5	+		Cocci
T6	-	+	Bacilli
T7	-	+	Bacilli
T8	-	+	Bacilli
T9	-	+	Bacilli
T10	-	+	Bacilli

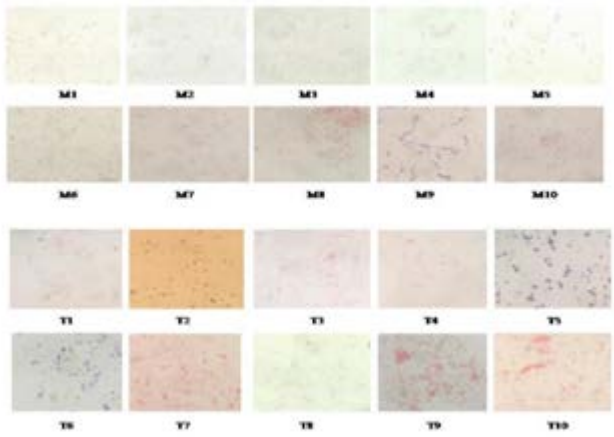

Fig. 2. Colony morphology and Gram staining of the isolates

1.4.3 TSI test:

The samples were observed after incubation for 18-20 hours at 37 °C. Isolates M1, M2, M3, M4, M5, M6, M8 of sample 1 and T2, T6, T7, T8, T9 of sample 2 were observed to have red butt-red slant which indicated that these samples cannot ferment glucose, lactose and sucrose and hence as TSI negative. The isolates M7, M9, M10 of sample 1 and T3, T4 isolates of sample 2 showed yellow butt-red slant and are glucose fermenting. Yellow butt-yellow slant was observed for isolates T1 and T5 of sample 2 indicating lactose or sucrose fermenting. 7 out of 20 isolates were found to be TSI positive.

1.4.4 Methyl Red Test:

After incubation at 37°C for 48 hours, methyl red was added into each inoculation. The isolate T1 of sample 2 and M3 M6, M9, M10 of sample 1 gave bright red colour that indicated a pH of 4.2 or less and hence the samples are methyl red positive. The isolates T2, T3, T4, T5, T6, T7, T8, T9, T10of sample 2 and M1, M2 isolates of sample 2 showed yellowish orange colour indicating they were methyl red negative. Isolates T5 of sample 2 and M4, M5, M7, M8 of sample 1 presented reddish orange that indicated weak methyl red positive.

1.4.5 VP Test:

The Voges-Proskauer test detects the presence of acetoin, a precursor of 2,3 butanediol. Isolates M3, M4, M5, M9 of sample 1 and the isolate T5 of sample 2 gave a pink-crimson colour that indicated the presence of acetoin and hence were VP positive.

1.4.6 Catalase and oxidase assay:

Isolates M1, M3, M4, M6, M7, M8of sample 1 and T1, T2, T3, T10 of sample 2 gave positive results that indicated these bacteria producing catalase enzyme that rapidly degraded H_2O_2. The isolates M3, M9, M10 of sample 1 and T1 of sample 2 gave oxidase positive results. This indicated that these samples produced cytochrome oxidase enzyme that catalysed the oxidation of reduced cytochrome by molecular oxygen forming H_2O or H_2O_2.

1.4.7 Citrate Assay:

In absence of glucose or lactose, some microbes use citrate as carbon source which depends on presence of citrate permease enzyme. Isolates M1, M2, M3, M4, M5, M6, M7, M8, M10 of sample 1 showed blue colour that indicated these microbes were citrate positive due to their ability to produce citrate permease enzyme, whereas all isolates of sample 2 were observed to be citrate negative. The summary of the results of biochemical test carried out for the isolates are presented in Table 3 and Fig 3.

Table 3: Summary of biochemical tests performed and identified bacteria

Isolates	Catalase test	Oxidase test	TSI assay	Methyl red test	Citrate assay	VP test	Probable bacteria
M1	Positive	Negative	Negative	Negative	Positive	Negative	*Vibrio gazogenes*
M2	Negative	Negative	Negative	Negative	Positive	Negative	*Chrysomonas luteola*
M3	Positive	Positive	Negative	Positive	Positive	Positive	*Vibrio cincinnatienses*
M4	Positive	Negative	Negative	Positive	Positive	Positive	*Vibrio metschnikovii*
M5	Negative	Negative	Negative	Positive	Positive	Positive	*Staphylococcus intermedius*
M6	Positive	Negative	Negative	Positive	Positive	Negative	*Vibrio gazogenes*
M7	Positive	Negative	Positive	Positive	Positive	Negative	*Flavimonas oryzihabitans*
M8	Positive	Negative	Negative	Positive	Positive	Negative	*Vibrio gazogenes*
M9	Negative	Positive	Positive	Positive		Positive	*Micrococcus mucilaginous*
M10	Negative	Positive	Positive	Positive	Positive		*Salomonella sp.*
T1	Positive	Positive	Positive	Positive	Negative	Negative	*Micrococcus luteus*
T2	Positive	Negative	Negative	Negative	Negative	Negative	*Staphylococcus simulans*
T3	Positive	Negative	Positive	Negative	Negative	Negative	*E. coli*
T4	Negative	Negative	Positive	Negative	Negative	Negative	*Photobacterium angustum*
T5	Negative	Negative	Positive	Positive	Negative	Positive	*Steptococcus group E*
T6	Negative	Negative	Negative	Negative	Negative	Negative	*Pseudomonas mallei/ Salmonella paratyphi*
T7	Negative	Negative	Negative	Negative	Negative	Negative	*Salmonella Paratyphi/ Pseudomonas mallei*
T8	Negative	Negative	Negative	Negative	Negative	Negative	*Pseudomonas mallei/ Pseudomonas paratyphi*
T9	Negative	Negative	Negative	Negative	Negative	Negative	*Salmonella paratyphi/ Pseudomonas mallei*
T10	Positive	Negative	Negative	Negative	Negative	Negative	*Klebsiella pneumoniae*

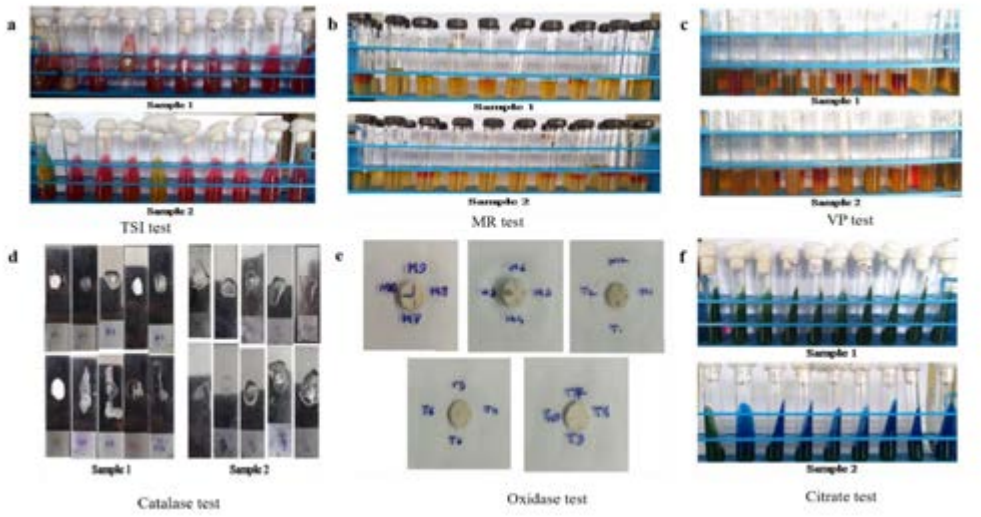

Fig 3. Representative pictures of the biochemical tests

1.4.8 Antibiotic Succeptibility by Kirby's disk diffusion assay:

The antibiotic diffusion plates were observed for the zone of inhibition after 24 hrs incubation. The summary of the susceptibility of the isolates to various antibiotics is presented in Table 4 and Fig. 4.

Table 4: Antibiotic disk diffusion assay results

Isolates	Ciprofloxacin (5mg)	Penicillin G (10mg)	Ceftizoxime (30 mg)	Gentamycin (10 mg)	Vancomycin (10 mg)
T2	S*	R*	R	R	S
T4	S	R	I*	S	I
T7	S	R	S	S	I
M3	S	R	R	S	S
M9	S	R	R	I	R

*S: Susceptible * I: Intermediate *R: Resistant

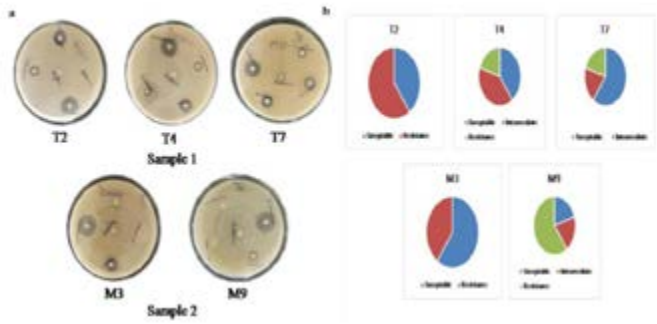

Fig 4. Antibiotic disc diffusion assay (a) and the pie chart showing the susceptibility pattern of the isolates to various antibiotics used in the study (b).

1.5 **Conclusion**

Various genera of bacteria were presumptively identified from Sample 1 and Sample 2. Pathogenic strains such as *Vibrio gazogenes, Chrysomonas luteola, Flavimonas oryzihabitans, Micrococcus luteus, Salmonella* sp. *and Salmonella paratyphi* which are known to cause diseases such as vibriosis, cholera, sepsis, bacteraemia, typhoid, pneumonia and others have been presumptively identified.

Among the isolates, T2 of sample 2 and M9 of sample 1 were found to be resistant to 3 different antibiotics. The isolate T2 of sample 2 was found to be resistant to penicillin, ceftizoxime and gentamycin and the isolate M9 of sample 1, a Gram-positive bacterium was found to be resistant to vancomycin, ceftizoxime and penicillin with gentamycin showing little to no effect. Four out of five isolates tested for antibiotic resistant were found to be resistant to penicillin. The isolate M9 of sample 2 has the potential to cause serious diseases as vancomycin resistant attribute makes it a formidable strain. Further research is required to quantitatively asses their characteristics. The possible diseases that may be caused by the bacteria isolated from sample 1 and sample 2 is provided in Table 5.

Table 5: Bacterial isolates identified and the possible related diseases

Bacteria	Diseases
Vibrio gazogenes	Vibriosis and cholera
Chrysomonas luteola	Bacteraemia, meningitis, prosthetic valve endocarditis, peritonitis
Vibrio metschnikovii	Peritonitis, inflamed gall bladder, cholecystitis
Staphylococcus intermedius	Urinary tract infection, bacteraemia in dogs
Flavimonas oryzihabitans	Sepsis, peritonitis, endophthalmitis, bacteraemia
Micrococcus mucilaginous	Bacteraemia, endocarditis, ventriculitis, peritonitis, pneumonia, endophthalmitis, keratolysis, septic arthritis..
Salomonella sp.	Fever, abdominal pain, diarrhoea, nausea and sometimes vomiting.
Micrococcus luteus	Septic shock
Staphylococcus simulans	Cardiac or osteoarticular infections in dogs
E. coli	Urinary tract infections, respiratory illness and pneumonia
Photobacterium angustum	Pathogen of marine life
Streptococcus group E	Soft tissue infections such impetigo and cellulitis.
Pseudomonas mallei/ Salmonella paratyphi	Typhoid fever
Klebsiella pneumoniae	Urinary tract infection (UTI), pneumonia, intra-abdominal infection, bloodstream infection (BSI), meningitis and pyogenic liver abscess (PLA)

It is important to understand the vast presence of microbes in everyday objects. Creating awareness about hygiene and the lurking dangers present in everyday objects is of utmost importance especially in times of pandemic as secondary infections by opportunist pathogens can be lethal apart from the dangers of primary infection existing in an host.

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