

Condoms-As Sources of Extracellular Enzyme Producing Microbes

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Abstract: Problem statement: Condoms are widely used birth control measures which are made of natural latex or synthetic materials like polyurethane or polyisoprene as matrix with lubricants added to make them ready to use commodities. Absence of sterilization method along with presence of lubricant like petroleum jelly (that are applied on the outer surface), prompted the investigators to look for microbes from the surface of male condoms. **Approach:** The culture based method was used for the study. This study reports the isolation and preliminary characterization of microbes from unused new condoms of ten different brands available in the chemist's shop in Kolkata, India. **Results:** The microbial count varies between $0-3 \times 10^{10}$ in samples obtained from different batches. Thirty four microbial isolates were selected for their colony and cell morphology as well as biochemical characterization. Ten among them were picked up for antibiotic sensitivity testing, quorum sensing behaviour and swimming and swarming behavior while six of them were characterized at the molecular level (16S rDNA). Most of the isolates were lipase producing. 10Gray of ⁶⁰Co gamma ray was found to be effective to decontaminate the condoms. **Conclusion/Recommendations:** The growth due to contamination might be supported by the lubricant. Hence it is time to consider seriously the need to maintain aseptic conditions during manufacturing.

Key words: Male condom, molecular identification, lubricant, quorum sensing, swarming behavior, extracellular enzyme producing microbes, petroleum jelly, Phosphate Buffer Saline (PBS), Colony Forming Unit (CFU)

INTRODUCTION

The use of glans condom as a means of contraception in Asia dates back to before the fifteenth century as per the historical records. Initially they were made of oiled silk paper or lamb intestine as in China, while of tortoise shell or animal horn as in Japan (Collier, 2007; Saravanamurthy *et al.*, 2010). There was opposition to the use of condoms yet it gained popularity as well as market and by eighteenth century different varieties of these were available (http://en.wikipedia.org/wiki/Condom#cite_ref-collier_0-48, 21st April 2010). Until early nineteenth century it was a commodity only for the affluent class but thereafter it was promoted to all sections of the society. Quality control in terms of leak proof testing was becoming common. The patent by Charles Goodyear in 1844 on the process of rubber

vulcanization revolutionized the condom market (<http://www.gutenberg.org/etext/14009>, http://www.goodyear.com/corporate/history/history_story.html, 21st April 2010). By 1855 the first rubber condom was produced (<http://www.billy-boy.com/english/info/>, 21st April 2010). Around 1912 Julius Fromm, a Jewish entrepreneur and chemist, the inventor of a process for making condoms from liquified rubber further revolutionized their manufacturing technology. The condoms prepared of latex now had a longer shelf life as compared to the older ones made of rubber. Its market doubled by 1920s. In those days each piece had to be handled manually by semiskilled workers. Another revolution in this field initiated during the decade (1920s) was the automation of the manufacturing unit (condom assembly line). A milestone in the path was the patenting in 1930 of a fully automated line. The quality

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regulations were made more stringent during 1960s and 1970s (http://en.wikipedia.org/wiki/Condom#cite_ref-collier_0-48, 21st April 2010), mostly concerning the leak proof nature. As per the 2006 annual report of the Tariff Commission 1.9 billion condoms were purchased in 2004 by the government of India for distribution at family planning clinics (<http://tc.nic.in/areports/annualreport-2005-06.pdf>, 21st April 2010).

Though the latex condoms had advantages over the rubber condoms, but there were certain drawbacks. They could not be used in association with lubricants like petroleum jelly and were also responsible for causing latex allergy. The latter reason was the sole cause for the alternative material of choice for condom preparation. Vytex which is 90% protein free latex and synthetic matrix like Polyisoprene and Polyurethane were the substitutes for latex condoms which could also be used in association with lubricants (http://www.vytex.com/BizDocs/REnvyFDA_Clearance.pdf, <http://www.vytex.com/Consumers/howvytexworks.aspx?pageid=C8>, http://healthnewsdigest.com/news/New_Product_460/Lifestyles_Condoms_printer.shtml, 21st April 2010). Some report talks about the role of the spermicidal lubricant as the source for causing UTI (<http://www.consumerreports.org/cro/cu-press-room/pressroom/archive/>, 21st April 2010).

The absence of any microbiological testing report, presence of lubricant and report of higher incidences of bacterial vaginosis in sexually active women (http://www.stdsservices.on.net/std/bacterial_vaginosis/facts.htm, 21st April 2010) prompted the group to look into the microbial quality of male condoms with special emphasis on lipase producing bacteria, which might be the carbon source for growth of the microbes on the condom surface.

MATERIALS AND METHODS

Microbial colony count from condom surface: Minimum five samples were taken for all ten Indian brands (Variety 1-10) tested. Each condom was aseptically transferred to sterile 15 mL Phosphate Buffer Saline (PBS) in 50 mL sterile falcon tubes, mixed vigorously using a vortex (CM 101 Cyclo Mixer, Remi Equipment Pvt. Ltd.) at maximum speed for five minutes and finally 50 µL suspension was spread on Luria Bertani (LB) agar plates. A negative control was set up treating PBS similarly without addition of the condom. Colony Forming Unit (CFU) count (Swe *et al.*, 2009; Schrapp and Al-Mutairi, 2010; Khatun *et al.*, 2009; Hawk *et al.*, 2010; Beers *et al.*, 2010; Ariful-Islam *et al.*, 2009) was taken post overnight incubation at 37°C under inverted condition and the total count per condom was determined. Two different brands from

another country were also tested similarly (Variety 11 and 12).

Morphological characterization: The isolates obtained post overnight incubation were compared morphologically by simple staining. The standard protocol of simple staining (Cappuccino and Sherman, 2007; Maripandi and Al-Salamah, 2010) was adopted for characterization of the thirty four isolates obtained through the initial spread plate method using bright field microscopy (Zeiss Axiostar Plus). The colony morphology, margin as well as colour for each of them were monitored.

Biochemical characterization: Qualitative tests for Protease (Georgalaki *et al.*, 2002, Mubarik *et al.*, 2010), DNase, Lipase, Oxidase, Catalase and Lecithinase were performed for all the thirty four isolates as per manufacturer's protocol (<http://www.himedialabs.com/TD/M1041.pdf>, <http://www.himedialabs.com/TD/M157.pdf>, http://www.himedialabs.com/ad_HiChrome.aspx, <http://www.himedialabs.com/resources/BioIDTestKitLow.pdf>). Moreover lipase quantification was done for four randomly selected isolates, by colorimetric estimation using para Nitro Phenol Palmitate (pNPP, Sigma Aldrich) as substrate (Sarkar *et al.*, 2008; Al-Quadan *et al.*, 2009). Protease was quantified by using spectrophotometric method as reported by (Malathu *et al.*, 2008; Mubarik *et al.*, 2010).

Antibiotic sensitivity profile: The sensitivity against different antibiotics was checked further for ten of the isolates as per the method reported by Nandy *et al.* (2007) and Abu-Al-Basal (2009).

Swarming and swimming behavior: These behaviors represent the colonizing property of the microbes and under such conditions microbes display entirely different properties as compared to their planktonic counterpart (Syafrullah and Salim, 2010). Antibiotic resistance was studied under both the motility conditions in semisolid LB agar plates (0.25% for swim and 0.5% for swarm plate) as per the protocol of (Lai *et al.*, 2009).

Quorum sensing property: The pure isolates inoculated as streaked zig zag lines close to each other on LB agar plates (1.5% agar) were incubated at 37°C for 48 hours. Post incubation their migration towards each other was observed and documented.

Molecular identification of the microbes: The molecular identification was based on 16S rDNA based characterization. The detailed steps followed for the molecular characterization were as reported by (Adarsh *et al.*, 2007; Nejat *et al.*, 2009; Al-Shami *et al.*, 2009).

Sterilization of contraceptive samples: Multiple samples of Variety 9 and 10 were irradiated at a dose of 10 Grays of ⁶⁰Co gamma rays. The microbial count pre and post irradiation for each variety was checked post vortexing at maximum speed in 15 mL PBS and subsequent plating on LB agar plates as already mentioned in the above section.

RESULTS

Detection and enumeration of microbes obtained from condom surface: Among the ten different Indian varieties checked, bacterial count were found to vary within $0-3 \times 10^{10}$ while that for the foreign variety varied between 0 to 10^2 CFU/sample (Table 1).

Morphological characteristics: Thirty four bacterial isolates were picked up at random from among the first ten different varieties of condoms and the microbes were classified based on the colony features, cell morphology and biochemical characteristics (Table 2). They were all circular with mostly regular margin (with few exceptions); yellow, white or cream in colour; cocci (either isolated or in cluster) or bacilli (isolated or in chain). The result depicted the diversity in the population with *Staphylococcus* forming the majority.

Biochemical characteristics: All the thirty four isolates were Catalase positive; thirty of them were Protease positive; Lipase was secreted by twenty three; Oxidase was present in seven of them; DNase was present in nine; while Lecithinase, the enzyme associated with pathogenic manifestation under clinical condition (Nandy *et al.*, 2009), was present in three of the isolates, as evident from the clearing zones in egg-yolk supplemented media (Yamashiro *et al.*, 2009). The isolates were found to secrete variable amounts of lipase enzyme: SRC_MC3 – 0.04 unit/ml; SRC_MC4-0.42 unit/ml; SRC_MC5 – 5.99 unit/ml; SRC_MC6-.23 unit/ml. Similarly their protease activity was found to be 2.49, 0.33, 1.83 and 0.66 unit/ml respectively.

Response towards different antibiotics: The antibiotic sensitivity profile of the isolates was important from the point of incurring the treatment in case of pathogenicity caused by them. As depicted in Table 3, the pure isolates exhibited mixed response towards the different groups of antibiotics. All the strains were found sensitive to gentamycin, doxycycline and tetracycline. Most of the strains were found to be sensitive to roxythromycin, a drug with a better cellular penetration. Other antibiotics which were found to be effective were neomycin, norfloxacin, ciprofloxacin and chloramphenicol.

Table 1: The microbial count obtained from twelve different varieties of condoms available in Indian (1-10) and foreign (11-12) market

Variety	Sample	Cell count
Variety 1	Sample 1	8.5×10^{-3}
	Sample 2	8×10^{-3}
	Sample 3	1.075×10^{-4}
	Sample 4	1.75×10^{-3}
	Sample 5	3.75×10^{-3}
Variety 2	Sample 1	4×10^{-3}
	Sample 2	1×10^{-3}
	Sample 3	0.5×10^{-3}
	Sample 4	0.5×10^{-3}
	Sample 5	0
Variety 3	Sample 1	1×10^{-3}
	Sample 2	1×10^{-3}
	Sample 3	1.5×10^{-3}
	Sample 4	0.5×10^{-3}
	Sample 5	0.5×10^{-3}
Variety 4	Sample 1	0.98×10^{-3}
	Sample 2	5×10^{-3}
	Sample 3	5.14×10^{-4}
	Sample 4	2.4×10^{-3}
	Sample 5	4.5×10^{-3}
	Sample 6	0
Variety 5	Sample 1	1.93×10^{-4}
	Sample 2	0.6×10^{-3}
	Sample 3	1.2×10^{-3}
	Sample 4	3×10^{-10}
	Sample 5	0.3×10^{-3}
	Sample 6	0
	Sample 7	0
	Sample 8	0
Variety 6	Sample 1	0.3×10^{-3}
	Sample 2	1×10^{-10}
	Sample 3	1×10^{-3}
	Sample 4	1.5×10^{-3}
	Sample 5	1.7×10^{-3}
	Sample 6	0
	Sample 7	0
	Sample 8	0
Variety 7	Sample 1	3×10^{-10}
	Sample 2	3×10^{-10}
	Sample 3	1×10^{-3}
	Sample 4	7.5×10^{-9}
	Sample 5	0.9×10^{-3}
	Sample 6	0
Variety 8	Sample 7	0
	Sample 1	3×10^{-10}
	Sample 2	3×10^{-10}
	Sample 3	3×10^{-10}
	Sample 4	5×10^{-3}
	Sample 5	1×10^{-10}
	Sample 6	0
Variety 9	Sample 7	0
	Sample 1	3×10^{-10}
	Sample 2	0.6×10^{-3}
	Sample 3	0.6×10^{-3}
	Sample 4	1.5×10^{-3}
	Sample 5	9.3×10^{-3}
	Sample 6	0
Variety 10	Sample 7	0
	Sample 1	3×10^{-10}
	Sample 2	4.08×10^{-5}
	Sample 3	3×10^{-10}
	Sample 4	2.4×10^{-3}
	Sample 5	1.11×10^{-4}
Variety 11	Sample 6	0
	Sample 7	0
	Sample 1	0
	Sample 2	0
	Sample 3	0
	Sample 4	0
Variety 12	Sample 5	0
	Sample 6	0
	Sample 1	6.66×10^{-1}
	Sample 2	1.33×10^{-2}
	Sample 3	0
	Sample 4	6.66×10^{-1}
Sample 5	0	
Sample 6	0	

Table 2: Table representing the characteristics of 34 isolates from the 10 different varieties of condoms. The different microbes obtained were classified based on their colony characteristics like color, margin, shape and morphology. Biochemical characterization were also done showing the presence of protease, catalase, oxidase, lipase and lecithinase enzymes. (+) denotes the presence of the enzyme while -denotes its absence

Sr.No.	Isolate	Shape	Margin	Color	Morphology	Lipase	Catalase	Oxidase	Protease	DNase	Lecithinase
1	SRC_MC1	circular	regular	yellow	staphylococci	No growth	+	-	+	+	-
2	SRC_MC2	circular	regular	cream	bacilli	No growth	+	-	+	+	-
3	SRC_MC3	circular	regular	cream	bacilli	+	+	-	+	+	-
4	SRC_MC4	circular	regular	white	bacilli with halo at the center	+	+	+	+	+	+
5	SRC_MC5	circular	regular	white	staphylococci	+	+	+	+	+	-
6	SRC_MC6	circular (very small)	regular	white	streptobacilli	+	+	-	+	+	-
7	SRC_M7	circular	serrated	white	bacilli	+	+	+	+	+	-
8	SRC_MC8	circular (very small)	regular	white	staphylococci	+	+	-	+	-	-
9	SRC_MC9	circular	regular	yellow	staphylococci	+	+	-	+	+	-
10	SRC_MC10	circular	regular	cream	cocci	+	+	+	+	+	-
11	SRC_MC11	circular	regular	white	staphylococci	+	+	-	+	-	-
12	SRC_MC 12	circular (large)	regular	yellow	staphylococci	-	+	+	-	-	-
13	SRC_MC 13	circular (large)	regular	white	cocci	+	+	-	+	-	-
14	SRC_MC 14	circular	regular	yellow	staphylococci	-	+	-	-	-	-
15	SRC_MC 15	circular	regular	white	cocci	+	+	-	+	-	-
16	SRC_MC 16	circular	regular	white	cocci	+	+	-	+	-	-
17	SRC_MC 17	circular (very small)	regular	yellow	staphylococci	-	+	-	+	-	-
18	SRC_MC 18	circular	regular	yellow	cocci	+	+	-	+	-	-
19	SRC_MC 19	circular (very small)	regular	white	staphylococci	+	+	-	+	-	+
20	SRC_MC 20	circular	serrated	cream	cocci	+	+	-	+	-	-
21	SRC_MC 21	circular	regular	cream	staphylococci	+	+	-	+	-	-
22	SRC_MC 22	circular	regular	yellow	cocci	+	+	-	+	-	-
23	SRC_MC 23	circular	regular	yellow	staphylococci	-	+	-	+	-	-
24	SRC_MC 24	circular (very small)	regular	white	staphylococci	-	+	-	+	-	-
25	SRC_MC 25	circular	regular	yellow	cocci	-	+	-	+	-	-
26	SRC_MC 26	circular (small)	serrated	yellow	bacilli	+	+	+	+	-	-
27	SRC_MC 27	circular	regular	white	bacilli	+	+	-	-	-	-
28	SRC_MC 28	circular (very small)	regular	cream	staphylococci	+	+	-	+	-	+
29	SRC_MC 29	circular (small)	serrated	yellow	cocci	-	+	+	+	-	-
30	SRC_MC 30	circular (very small)	regular	yellow	cocci	-	+	-	+	-	-
31	SRC_MC 31	circular	regular	cream	cocci	-	+	-	+	-	-
32	SRC_MC 32	circular	regular	yellow	cocci	-	+	-	+	-	-
33	SRC_MC 33	circular	regular	yellow	cocci	-	+	-	-	-	-
34	SRC_MC 34	circular	regular	yellow	staphylococci	+	+	-	+	-	-

Table 3: The antibiotic sensitivity of 10 different isolates based on the diameter of the clearance zone as compared to the standard chart provided by National Committee for Clinical Laboratory Standard's (NCCLS). In the above table 'S' denotes sensitive, 'R' denotes resistant and 'I' denotes intermediate response. The abbreviations for the antibiotics are as follows: A for Ampicillin, Cq for Cephadroxil, C for Chloramphenicol, Cx for Cloxacillin, Ce for Cephotaxime, Ca for Ceftazidime, Cf for Ciprofloxacin, Do for Doxycycline Hydrochloride, G for Gentamicin, Mt for Metronidazole, N for Neomycin, Nx for Norfloxacin, Pb for Polymyxin B, R for Rifampicin, Ro for Roxithromycin, T for Tetracycline, Tr for Trimethoprin, Va for Vancomycin

Antibiotic	SRC_MC1	SRC_MC2	SRC_MC3	SRC_MC4	SRC_MC5	SRC_MC6	SRC_MC7	SRC_MC8	SRC_MC9	SRC_MC10
G	S	S	S	S	S	S	S	S	S	S
N	S	S	S	S	R	S	S	S	S	S
Cq	S	S	R	S	S	S	S	S	I	R
Ce	S	R	S	R	R	I	I	S	R	I
Ca	S	R	R	R	R	R	R	R	R	R
Va	S	S	S	R	R	S	S	S	S	S
Nx	S	S	S	S	I	S	S	S	S	S
Cx	S	R	R	R	R	R	R	S	R	R
Pb	S	R	I	R	R	R	I	S	I	I
A	S	R	R	R	R	R	R	S	R	S
Cf	S	S	S	S	I	S	S	S	S	S
Ro	S	S	S	S	I	S	S	S	S	S
Do	S	S	S	S	S	S	S	S	S	S
R	S	R	S	I	I	S	S	S	R	I
Tr	S	R	S	R	R	R	R	S	R	R
T	S	S	S	S	S	S	S	S	S	S
Mt	R	R	R	R	R	R	R	R	R	R
C	S	S	S	S	I	S	S	S	S	I

Table 4: Differential response of isolates towards the antibiotic gentamycin under different motility conditions. For solid plate, the log phase culture was diluted 100 times and was plated on LB agar plates containing 1.5% agar. In case of swim plates (0.25 % agar) and swarm plates (0.5% agar), inoculation was spotted on one end of the plate and antibiotic disc was placed on the opposite end. In all cases the plates were incubated for overnight at 37°C and thereafter the diameter of clearance was measured

Isolate	Zone diameter in case of solid plates (cm)	Zone diameter in case of swim plates (cm)	Zone diameter in case of swarm plates (cm)
SRC_MC5	2.0	3.2	3.1
SRC_MC6	1.5	2.2	2.5
SRC_MC7	2.1	2.6	3.0
SRC_MC9	2.0	3.0	3.1
SRC_MC10	2.0	2.0	Colony did not grow

Swarming and swimming behavior pattern: These motility patterns are a result of the interaction between the bacterial communities. In this case the motility behavior was studied in correlation with the antibiotic sensitivity of the isolates. Five isolates exhibited swimming and swarming property when checked in presence of antibiotic gentamycin. This particular antibiotic was selected because all the isolates showed maximum sensitivity towards it in solid LB plates (1.5% agar). Figure 1 depicts the motility pattern in case of isolate SRC_MC5 where there was clear depiction of secondary migration fronts in both cases which could be due to selection of subpopulation of resistant microbes. In case of antibiotic gentamycin, the cells under swimming and swarming condition exhibited more sensitivity as compared to those plated on solid medium containing 1.5% agar (Table 4). Such responses are important for deciding the treatment procedure in case of infection.

Quorum sensing property: In this study quorum sensing property of the isolates was checked in form of mutual inhibition of growth by the various isolates which could be due to secretions that were lethal beyond a critical threshold level. As observed in Fig. 2a and 2b, this inhibition was observed in 6 cases between isolates SRC_MC1 and SRC_MC2, SRC_MC3 and SRC_MC5, SRC_MC2 and SRC_MC7, SRC_MC3 and SRC_MC10, SRC_MC4 and SRC_MC10 as well as SRC_MC2 and SRC_MC4.

Molecular identification of the microbes: The molecular identification was done for six of the ten pure isolates, tested for antibiotic sensitivity. All the isolates were found to be novel with 99% identity with other known bacteria and were found to belong to family *Bacillaceae*. Their GenBank accession numbers were: GU374111, GU374113, GU374115, GU374117, GU374119, GU374121.

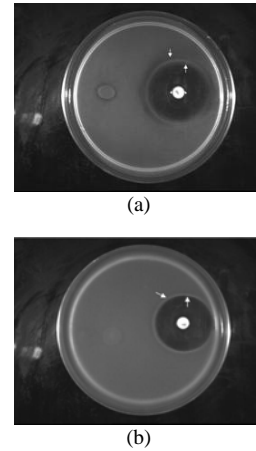


Fig. 1: Figure depicting the response of isolate SRC_MC5 towards antibiotic gentamycin under different motility condition (a) Photograph depicting swimming motility and (b) swarming motility of isolate SRC_MC5. The antibiotic disc (Gentamycin) was placed opposite to the point where the strain was inoculated as a point. The outer arrow indicates primary front and inner arrow shows the secondary migration front

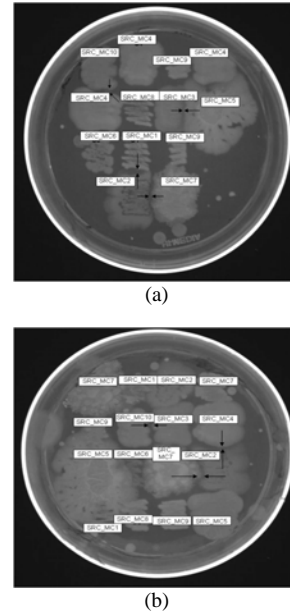


Fig. 2a and b: Figure showing the quorum sensing property of different isolates. The isolates were inoculated as zig zag lines close to each other. The plates were incubated at 37°C for overnight and the quorum sensing property was observed in form of mutual inhibition of growth as indicated by the black arrows

Sterilization of contraceptive samples: Un-irradiated samples of variety 9 showed a count varying between 6.2×10^4 to 1×10^8 ; while variety 10 showed a variation from $0-1 \times 10^8$. There was complete inhibition of growth in irradiated samples. Thus, ^{60}Co gamma irradiation helps remove microbial contamination from unsterilized male condoms.

DISCUSSION

Under most of the cases there was bacterial population present on condoms which would enter the female vaginal passage during the course of their application. Since, the manufacturing date of the samples tested varied between May 2008 and December 2009, the variation in count within the same variety as well as among the different varieties might be influenced by the manufacturing date/shelf life (Singh and Pandey, 2010) of the condoms. Two different brands from another country was also tested.

The prevalence of *Staphylococcus* was evident from both simple staining data as well as the black pigmentation on the Lecithinase detection plates. *Staphylococcus* in general and *Staphylococcus saprophyticus* (Peggy *et al.*, 1980; Gillespie *et al.*, 1978; Saravanamurthy *et al.*, 2010; Devieux *et al.*, 2009; Malow *et al.*, 2009) in particular are known to be associated with UTI in case of sexually active women (http://www.ehow.com/about_5449992_staphylococcus-saprophyticus-urinary-tract_infection.html).

The biochemical characterization reveals the presence of various enzymes in the isolates. Presence of Oxidase enzyme denotes their ability to catalyze an oxidation reduction reaction involving molecular oxygen as the electron acceptor. Presence of catalase indicates their role in protection of the cells from oxidative stress. It is a common feature for the aerobic microbes. Protease secretion in microbes can have two major purposes namely (a) degradation of the surrounding microbes as well as proteinaceous material in the environment to facilitate access to nutrition in a competitive surrounding and (b) to invade the periplasmic membrane in case of pathogens. Lipase secretion indicates lipid utilization as the carbon source for their growth. This could possibly indicate towards the sustenance of microbial growth by the lubricant present on the condom surface.

The antibiotic profile illustrates the sensitivity pattern of the isolates to various classes of antibiotics. Gentamycin is an aminoglycoside and is mainly directed for treating infection caused by aerobic gram negative bacteria like *Pseudomonas*, *Acinetobacter* and *Enterobacter*. Doxycycline and tetracycline belongs to

same group of antibiotic and they can be effectively used in treatment of such infections. Norfloxacin and ciprofloxacin were third generation antibiotics and they were reported to be frequently used for treatment of urinary tract infections. Thus, the antibiotic profile of the isolates would guide the treatment procedure in case of infection.

Since, it has already been reported that under different motility conditions the bacterial population is reported to exhibit different morphological and physiological characteristics and the primary objective of this study was to understand the differential response towards antibiotic. Swarming and swimming are typical surface translocation phenomenon of bacteria. These motility patterns also indicate the colonization property of the isolates and basically these depend on the surface feature and density of the medium. The swarming behavior might depend on the metabolite concentration or in this case antibiotic diffusion front on the solid surface. As in case of negative chemotaxis if the cell growth is inhibited by the secreted metabolite or antibiotic, the cells exhibit swarming phenomenon. The swarming movement is reported to be induced by development of peritrichous flagella and the migration of cells is facilitated by secretion of extracellular slime. The slime causes the aggregation of cells and develops a matrix which basically forms biofilm (Salamitou *et al.*, 2009). From clinical point of view, infection in hosts is mainly caused by biofilm communities. The establishment of biofilm can cause blockage in the path, resistance to antibiotics, or even septicemia.

Quorum sensing is another property that dictates the coordination between the bacterial colonies. Bacteria exhibiting quorum sensing property are known to secrete signaling molecules called autoinducers or pheromones. The activation of cellular receptors by the inducers directs the gene transcription and in other words controls the cellular behavior. Moreover lipase enzyme is expressed by microbes for quorum sensing regulated operon system and transported from cytoplasm to outer environment either via Sec-Dependent pathway or by ABC transporter (Rosenau and Jaeger, 2000; Ali *et al.*, 2010). Lipase positive SRC_MC3, SRC_MC4 and SRC_MC5 strains were found to show quorum sensing behavior. The colonies showing quorum sensing mutually inhibited the growth which could be due to competition for nutrition or because of certain inhibiting metabolite secreted by the cells. This study was also important to understand the cell behavior when they inhabit the surface inside the body. The mutual behavior would tell the possibilities of development of biofilm when these microbes co-inhabit inside the human body. One major challenge with biofilm formation is their insensitivity to

antibiotics which may complicate treatment in case of vaginosis.

As reported in US patent 5800542, the total dose of gamma rays for sterilization is generally in the range of 15kilo Gray to 35kilo Gray, however in our case 10 gray irradiation was found to be sufficient to decontaminate the condoms. This finding points towards the feasibility of using this method at a commercial scale.

CONCLUSION

Thus, this study highlights for the first time microbes in some of the commercially available male condoms in the Indian as well as foreign market leading to possibility of health hazard. The relative CFU in international brands were much less. This study also reflects the condom as source of extracellular protease and lipase producing microbes. As evident from the data in Table 1, if a person chooses a brand randomly, then the probability that the packet will have bacterial contamination is 75.4%. This is because out of 65 samples only 16 are not contaminated. In the light of this finding it might be necessary to consider seriously the maintenance of aseptic condition during the manufacturing of the condoms and in its delivery package. Keeping in mind the drastic decrease in colony count post irradiation, treatment of the packed condom samples with a low dose of Gamma rays (~10Gray) can be used as an efficient method for sterilization before distribution.

Disclaimer: This reflects the deep concern but does not in any way indict any specific manufacturer. The condoms were selected at random.

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