Effect of Microgravity on Most Frequently Isolated Microorganisms from Cosmetics

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Abstract: Microorganisms associated with commonly used cosmetics and effects of microgravity on most frequently isolated microorganism were investigated. The microorganisms isolated from the cosmetics were *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Bacillus cereus, Proteus vulgaris, Bacillus subtilis, Trichoderma piluliferum* and *Neocosmospora vasinfecta.* Fifty percent of the cosmetics were contaminated with *Staphylococcus aureus,* 31.82% contaminated with *Pseudomonas aeruginosa,* 22.73% contaminated with *Escherichia coli,* 13.64% contaminated with *Proteus mirabilis,* 13.64% contaminated with *Bacillus cereus,* 9.09% contaminated with *Proteus vulgaris,* 4.55% contaminated with *Bacillus subtilis,* 13.64% contaminated with *Trichoderma piluliferum* and 9.09% contaminated with *Neocosmospor avasinfecta.* The *S. aureus* which was the most frequently isolated bacteria was subjected to microgravity condition. The *S. aureus* grown under stimulated microgravity condition exhibited resistance to antibiotic more than under earth gravity. The resistance of the 5. *aureus* to antibiotics tends to increase with increased in revolution per minutes (rpm) at which the bacterium was subjected.

Keyword: Cosmetics, microorganisms, contamination, antibiotics, gravity.

Introduction

Preservatives are used in all sorts of products in which microorganisms can proliferate. Food, pharmaceuticals, industrial products, household products and cosmetics are some of the products that are at risk of contamination by microorganisms. Presently, the cosmetic industry uses numerous ingredients, including preservatives, moisturizers, thickeners, antimicrobials, solvents, emulsifiers and colours in the production processes. Pathogenic microorganisms such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* are frequently encountered in contaminated cosmetics (Lundov *et al.*, 2009).

Microbes are highly evolved and occupy unique environmental niches. In order for the plethora of microbes to persist and thrive, they must be highly responsive to change in their local environments in which they are constantly exposed. Some of these environmental changes are experienced in the form of temperature, oxidative, host cell induced, and/or chemical stresses (Resenzweig *et al.*, 2010). Microbial existence and survival requires the ability to sense and respond to environmental changes, including changes in physical forces as well as mutant availability and accessibility. This is because microbes inhabit an amazingly diverse range of ecological niches and therefore, must constantly adapt to a wide variety of changing environmental conditions, including alterations in temperature, pH, nutrient availability, oxygen levels, and osmotic pressure gradients (Foster and Spector, 1995; Cavicchioli *et al.*, 2000; Audia *et al.*, 2001; Hecker and Volker, 2001; Hengge-Aronis, 2002; Poolman *et al.*, 2002).

Recently, several important studies have demonstrated a key role for microgravity and the low fluid shear dynamics associated with microgravity in the regulation of microbial gene expression, physiology, and pathogenesis (Dickson, 1991; Mishra and Pierson, 1992; Kacena *et al.*, 1999; Klaus, 2002; Nickerson *et al.*, 2003). Diminished gravity or 'microgravity' as it is termed, weakens humans and makes bacteria stronger, more difficult to kill, and more accomplished at causing disease (Matin *et al.*, 2006).

Bone decalcification and loss are well documented in astronauts during space travel and residence. This predisposes them to bone fracture as well as kidney stones from resorbed bone material (Matin *et al.*, 2006). In microgravity, muscles atrophy and blood production decreases. The latter results in diminished pumping by the heart and, combined with the concomitant blood shift to the upper torso, can damage heart muscles (Matin *et al.*, 2006). Effect of microgravity on bacteria decreased antibiotics effectiveness against such bacteria.

The objective of this study was to examine the assessment of microorganisms associated with commonly used cosmetics and the effect of microgravity on the most frequently isolated microbe.

Material and Methods

Samples collection and storage

Twenty two different brands of cosmetics were randomly purchased from shops and drug stores at Ojaoba within Akure metropolis. The cosmetics included five lotions, two Vaseline, eight cream, five

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powder and two natural cosmetics. All the samples collected were stored in the refrigerator in the microbiology laboratory of The Federal University of Technology Akure, Nigeria. Prior to storage the samples were inspected for any physical defects and organoleptic characteristics. The container label information such as batch number, expiry date, manufacturing date, directions for use and composition, which should be disclosed as per the Good Manufacturing Practice Certification (GMPC), were recorded (The European Cosmetic Toiletry and Perfumery Association, 1994).

Microbiological assessment of the cosmetics

Aerobic plate count

Aerobic plate count was carried out as described by Food and Drug Administration, 1992, Mwambete and Simon (2010) with slight modification. The outside surface of each container was swabbed with 70% ethanol before opening. One gram of each of the cosmetics was serially diluted in physiological buffer solution of pH 7. A five-fold serial dilution was made and 0.1ml of the 10^{-3} and 10^{-5} dilutions were uniformly spread-plated onto 14 cm diameter wide agar plates on each of the solid media, Nutrient agar (NA), MacConkey agar (MCA) and Suboraud's dextrose agar (SDA) (Oxoid, UK) for detection of microbial contamination. The inoculated agar plates were aerobically incubated at 37°C for 24 hours for bacteria and at 27°C for 48 hours for fungi. The resultant colonies were counted and recorded as colony-forming units per gram of sample (cfu/g) for bacteria and spore forming unit per gram of sample (sfu/g) for fungi. Each sample was assayed in triplicate and the average value for cfu/g was calculated.

Anaerobic plate count (use only for talcs and powders).

Anaerobic plate count was done as describe by Hitchins *et al.* (2001).The main purpose of this procedure is to detect the tetanus bacillus (*Clostridium tetani*), which can occur in these products. Five-percent (5%) defibrinated sheep blood agar was use for plating. One gram of each of the cosmetics was serially diluted in physiological buffer solution of pH 7. A five-fold serial dilution was made and 0.1 ml of the 10^{3} and 10^{5} dilutions were uniformly spread-plated on 5% defibrinated sheep blood agar to minimize spreading of growth caused by wetness. The inoculated plates were placed in an anaerobic atmosphere within minutes after inoculation to minimize exposure to oxygen. The blood agar plates were incubate in 5-10% carbon dioxide atmosphere (CO_2 incubator) for 48 hours before counted. The plate that has no colonies after 48 hours was re-incubated for 2 more days for colony count observation.

Identification of microorganisms isolated from cosmetics

Identification of bacteria

Parameters used in differentiating each isolates include colonial characteristics (edges, texture, elevation, colour, pigmentation, and size, cell morphology (Shape, arrangement and Gram reaction). Bacterial isolated from each plated Petri dishes were subcultured onto selective agar (Salmonella Shigella agar, Eosin methylene blue agar) so as to differentiate enteric bacilli. The bacteria were further analyzed by conventional biochemical tests according to the methods described by Olutiola *et al.* (2000) and Cheesbrough (2010).

Identification of fungi

Fungal isolates were characterized and identified based on macrospcopic and microscopic details with reference to Barnett and Hunter (1998).

Standardization of test bacteria

A loopful of the bacterial culture was aseptically inoculated into freshly prepared sterile nutrient broth and incubated for 24 hours. Zero-point-Two was pipetted from the 24 hours broth culture of the test organism was dispensed into 20 ml sterile nutrient broth and incubated for another 4 hours to standardise the culture to 0.5 McFarland's standard (10^{6}cfu/ml) before use as described by Oyeleke *et al.* (2008).

Antibiotic sensitivity profile of the bacteria isolated from cosmetics

The antibiotic sensitivity profile was investigated in order to compare the sensitivity of the microorganisms to the different conventional antibiotics. The disc diffusion method was used to determine the susceptibility and resistance of the organisms to the antibiotics. Twenty milliliter of sterile Mueller-Hilton agar was aseptically poured into sterile Petri dishes and allowed to gel. Each plate was seeded with the test organism before aseptically introducing the antibiotic disc with sterile forceps onto the surface of the solidified Mueller Hilton agar plate and incubated at 37°C for 24 hours. After incubation, clear zones around the disk were measured in millimeter and recorded as the zones of inhibition. Diameters of zone of inhibition was measured with a calibrated ruler and then compared with clinical and laboratory standards institute standard for bacteria sensitivity or resistance to the antibiotic. Seeded plates without antibiotic disk served as the control. The antibiotic sensitivity profile was carried out in triplicates.

Microgravity of the most frequently isolated microorganism from cosmetics

The most frequently isolated microorganism was streak on a sterile nutrient agar plates and was subjected to microgravity. The most frequently isolated organism was subjected to

microgravity at 2 rmp for 5 minutes, 20 rmp for 10 minutes up to 30 minutes at 5 minutes interval using Clinostat and incubated for 24 hrs. The *in vitro* antibiotic susceptibility test of most frequent microorganism isolated from the cosmetics subjected to microgravity was done using disc diffusion method described by Bauer *et al.* (1996).

Statistical analysis of data obtained

Data obtained were subjected to one way analysis of variance, while the means were compared by Duncan's New Multiple Range Test at 95% confidence interval using Statistical Package for Social Sciences version 16.0. Differences were considered significant at $p \leq 0.05$.

Results

Table 1: Rate of occurrence of different bacteria isolated from cosmetics employ course of the study

Bacteria	Number of sample tested positive	Frequency distribution (%)
Staphylococcus aureus	11	34.38
Pseudomonas aeruginosa	7	21.88
Escherichia coli	5	15.63
Proteus mirabilis	3	9.38
Bacillus cereus	3	9.38
Proteus vulgaris	2	6.25
Bacillus subtilis	1	3.13
Total	32	100.03

The rate of occurrence of different bacteria isolated from cosmetics is presented in Table 1. *Staphylococcus aureus* was the most predominant bacteria isolated in the course of the research with frequency distribution (34.38%), followed by *Pseudomonas aeruginosa* (21.88%), *Escherichia coli* (15.63%), *Proteus Mirabilis* (9.38%), *Proteus vulgaris* (6.25%) and *Bacillus subtilis* (3.13%)

Table 2: Percentage of cosmetics contaminated with each of the bacteria isolates

Bacteria	Number of cosmetics tested positive	% positivity
Staphylococcus aureus	11	50.00
Pseudomonas aeruginosa	7	31.82
Escherichia coli	5	22.73
Proteus mirabilis	3	13.64
Bacillus cereus	3	13.64
Proteus vulgaris	2	9.09
Bacillus subtilis	1	4.55
Total number of cosmetics tested	22	

The percentage of cosmetics contaminated with each of the bacteria isolates is shown in Table 2.Fifty percent of the cosmetics were contaminated with *Staphylococcus aureus*, 31.82% of the cosmetics were contaminated with *Pseudomonas aeruginosa*, 22.73% of the cosmetics were contaminated with *Escherichia coli*, 13.64% of the cosmetics were contaminated with *Proteus mirabilis*, 13.64% of the cosmetics were contaminated with *Bacillus cereus*, 9.09% of the cosmetics were contaminated with *Proteus vulgaris* and 4.55% of the cosmetics were contaminated with *Bacillus subtilis*.

Table 3: Rate of occurrence of different fungal isolated from cosmetics employed in the course of the study

Fungi	Number of sample tested positive	Frequency distribution (%)
Trichoderma piluliferum	3	60
Neocosmospora vasinfecta	2	40
Total	5	100

The rate of occurrence of different fungi isolated from cosmetics is presented in Table 3. *Trichoderma piluliferum* was the predominant fungi isolated in the course of the research.

Table 4: Percentage of cosmetics contaminated with each of the fungal isolates

Fungi	*Number of cosmetics tested positive*	*(%) positivity*
Trichoderma piluliferum	3	13.64
Neocosmospora vasinfecta	2	9.09
Total number of cosmetics tested	22	

The percentages of cosmetics contaminated with each of the fungal isolates are shown in Table 4.Thirteen-point-six-four percent of the cosmetics were contaminated with *Trichoderma piluliferum* while 9.09% of the cosmetics were contaminated with *Neocosmospora vasinfecta*.

Table 5: Antibiotics sensitivity test (mm) on Gram positive and Gram negative microorganisms isolated from Cosmetics

Antibiotics	*S. aureus*	*B. subtilis*	*B. cereus*	*Pseu. aeruginosa*	*P.vulgaris*	*P. mirabilis*	*E. coli*
SXT	19.00±1.00 g	10.33±0.58^{f}	10.67±0.5f	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	7.33±0.58^{c}
CPX	16.33±0.58^{f}	11.33±0.58^{fg}	10.00±0.00^{ef}	0.00±0.00^{a}	6.33±0.58^{de}	5.33±0.58^{bcd}	7.33±0.58^{c}
AM	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00a	0.00±0.00a	8.67±0.58^{d}
CN	14.00±1.00^{e}	13.00±1.00^{hi}	13.67±0.58^{h}	0.00±0.00^{a}	0.00±0.00^{a}	6.33±1.15^{de}	13.00±1.00^{f}
PEF	16.33±0.58^{f}	8.00±0.00^{c}	9.00±1.00^{de}	0.00±0.00^{a}	0.00±0.00^{a}	6.67±0.58^{e}	10.67±1.15^{e}
S	12.67±1.15^{d}	8.33±.0.58^{e}	8.33±1.52^{d}	0.00±0.00^{a}	5.67±0.58^{cd}	4.67±0.58^{bc}	0.00±0.00^{a}
APX	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	11.00±1.00^{g}	0.00±0.00^{a}
Z	8.00±0.00^{b}	12.33±0.58^{gh}	13.67±0.58^{h}	0.00±0.00^{a}	7.67±0.58^{f}	8.67±0.58^{f}	0.00±0.00^{a}
R	16.33±0.58^{f}	12.67±1.15^{hi}	13.00±0.00^{gh}	0.00±0.00^{a}	5.33±0.58^{b}	4.33±0.58^{b}	4.67±1.15^{b}
E	12.33±0.58^{d}	13.67±0.58^{i}	12.00±0.00^{g}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
CH	11.00±1.00^{c}	7.67±0.58^{de}	5.33±0.58^{c}	0.00±0.00^{a}	4.33±0.58^{b}	0.00±0.00^{a}	8.33±0.58^{cd}
SP	10.67±0.58^{c}	5.00±0.00^{c}	3.33±0.58^{b}	0.00±0.00^{a}	0.00±0.00^{a}	5.67±0.58^{cde}	8.00±1.00^{cd}
AU	8.33±0.58^{b}	3.67±0.58^{b}	0.00±0.00^{a}	0.00±0.00^{a}	7.00±1.00^{ef}	14.00±1.00^{h}	8.67±0.58^{d}
OFX	12.67±1.15^{d}	6.67±1.53^{d}	5.00±1.00^{c}	4.67±0.58^{b}	8.67±0.58^{g}	13.67±0.58^{h}	8.67±0.58^{d}

Data are presented as Mean±S.D. Values with the same superscript letter(s) along the same column are not significantly different (P<0.05).

Key: SXT = Septrin (30ug), CPX = Ciprofloxacin (10ug), AM = Amoxicillin (10ug), CN = Gentamycin (10ug), PEF = Pefloxacin (30ug), S = Streptomycin (30ug), APX = Ampiclox (30ug), Z = Zinnacef (20ug), R = Rocephin (25ug), E = Erythromycin (10ug), CH = Chloramphenicol (30ug), SP = Sparfloxacin (10ug), AU = Augmentin (30ug), OFX = Tarivid (10ug)

The antibiotics sensitivity test on Gram positive and Gram negative microorganisms isolated from cosmetics are presented in Table 5. The microorganisms isolated from cosmetic were *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa, Proteus vulgaris, Proteus mirabilis* and *Escherichia coli. Pseudomonas aeruginosa* was the most resistant bacteria to antibiotics in the course of the research.

Table 6: Antibiotics resistance pattern (mm) of most frequently isolated organisms from cosmetics (*Stapylococcus aureus*) subjected to microgravity

Antibiotics	*S. aureus*	*S. aureus* @ 2rpm for 5 mins	*S. aureus* @ 20rpm for 10 mins	*S. aureus* @ 20rpmfor 15 mins	*S. aureus* @ 20rpm for 20 mins	*S. aureus* @ 20rpm for 25 mins	*S. aureus* @ 20rpm for 30 mins
SXT	19.00±1.00^{g}	18.67±1.15^{h}	10.33±0.58^{e}	10.00±0.00^{f}	10.00±0.00^{e}	9.67±0.58^{e}	9.67±0.58^{e}
CPX	16.33±0.58^{f}	16.00±0.00^{g}	13.00±1.00^{f}	12.33±0.58^{g}	12.33±0.58^{g}	12.00±0.00^{g}	12.00±0.00^{g}
AM	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
CN	14.00±1.00^{e}	6.67±1.15^{b}	6.33±0.58^{c}	6.33±0.58^{d}	6.00±0.00^{c}	4.33±0.58^{b}	4.33±0.58^{b}
PEF	16.33±0.58^{f}	16.33±0.58^{g}	12.33±0.58^{f}	12.33±0.58^{g}	12.00±0.00^{g}	12.00±0.00^{g}	10.67±1.15^{f}
S	12.67±1.15^{d}	12.33±0.58^{e}	8.33±0.58^{d}	8.33±0.58^{e}	6.33±0.58^{c}	6.00±0.00^{c}	6.00±0.00^{c}
APX	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Z	8.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
R	16.33±0.58^{f}	14.33±0.58^{f}	10.33±0.58^{e}	8.33±0.58^{e}	6.33±0.58^{c}	5.67±0.58^{c}	5.67±0.58^{c}
E	12.33±0.58^{d}	6.67±1.15^{b}	6.33±0.58^{c}	4.33±0.58^{c}	4.00±0.00^{b}	4.00±0.00^{b}	3.67±0.58^{b}
CH	11.00±1.00^{c}	6.33±0.58^{n}	6.33±0.58^{c}	6.00±0.00^{d}	6.00±0.00^{c}	5.67±0.58^{c}	5.33±1.15^{c}
SP	10.67±0.58^{c}	10.00±0.00^{d}	8.33±0.58^{d}	8.33±0.58^{e}	8.33±0.58^{d}	8.00±0.00^{d}	7.67±0.58^{d}
AU	8.33±0.58^{b}	8.33±0.58^{c}	2.67±1.15^{b}	2.33±0.58^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
OFX	12.67±1.15^{d}	12.33±0.58^{e}	12.33±0.58^{f}	12.33±0.58^{g}	11.00±1.00^{f}	10.33±0.58^{f}	10.00±0.00^{ef}

Data are presented as Mean±S.D. Values with the same superscript letter(s) along the same column are not significantly different (P<0.05).

Key: SXT = Septrin (30ug), CPX = Ciprofloxacin (10ug), AM = Amoxicillin (10ug), CN = Gentamycin (10ug), PEF = Pefloxacin (30ug), S = Streptomycin (30ug), APX = Ampiclox (30ug), Z = Zinnacef (20ug), R = Rocephin (25ug), E = Erythromycin (10ug), CH = Chloramphenicol (30ug), SP = Sparfloxacin (10ug), AU = Augmentin (30ug), OFX = Tarivid (10ug).

Antibiotics resistance pattern (mm) of most frequently isolated organisms from cosmetics (*Staphylococcus aureus*) subjected to microgravity are shown in Table 6. The *Staphylococcus aureus* was subject to microgravity *at* 2 rpm for 5 mins, 20 rpm for 10 mins, 20 rpm for 15 mins, 20 rpm for 20 mins, 20 rpm for 25 mins and 20 rpm for 30 mins. The bacteria grown under actual reduced gravity conditions

exhibited resistance to antibiotics more than they do under one gravity.

Discussion

Most of the cosmetics employed in the course of the study were contaminated with microorganisms. Up to 9.09% (2 out of 22) of the cosmetics were not contaminated with microorganisms, 90.91% (20 out of 22) of the cosmetics were contaminated with microorganisms. The isolated microorganisms included *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Bacillus cereus, P. vulgaris, B. subtilis, Trichoderma piluliferum* and *Neocosmospora vasinfecta.. Staphylococcus aureus* and *Pseudomonas. Aeruginosa* were the most frequent isolated bacteria from the cosmetics. This is in accordance with the report of Lundov *et al.* (2009) who reported that pathogenic microorganisms such as *S. aureus* and *Pseudomonas aeruginosa* are frequently found in contaminated cosmetics. *Staphylococcus aureus* were found to be the most predominant microorganisms and/or bacteria with highest frequency distribution (34.38%) isolated from cosmetics use in the course of the research. Fifty percent of the cosmetics were contaminated with *Staphylococus aureus.*

The bacteria grown under actual reduced gravity condition exhibited resistance to antibiotics. This is in line with the finding of Lynch *et al.* (2004) and Allen *et al.* (2008). Previous studies have shown that bacteria grown under either actual reduced gravity or Modeled Reduced Gravity (MRG) conditions, surprisingly, exhibited resistance to multiple antibiotics (Lynch *et al.*, 2004; Allen *et al.* 2008) and become more virulent, which has important potential impacts for human health (Nickerson *et al.*, 2000; Wilson *et al.*, 2007; Rosenzweig *et al.*, 2010). The resistance to antibiotic of bacteria grown under actual reduced gravity condition could be as a result of bacteria under these conditions have enhanced growth (Brown *et al.*, 2002; Kacena *et al.*, 1999; Mauclaire and Egli, 2010), secondary metabolite production (Demain and Fang, 2001), biofilm formation (McLean *et al.*, 2001) and extracellular polysaccharide production (Mauclaire and Eglil, 2010). The resistance of the *S. aureus* to antibiotics tends to increase with increased in revolution per minutes (rpm) at which the microorganism was subjected. This is in agreement with the report of Matin *et al.* (2006) who reported that a large body of work conducted with bacteria grown under conventional normal gravity conditions over the last two or so decades has shown that increased bacterial virulence is accompanied with increased resistance to antimicrobial stresses. The large-scale physiological effects of microgravity-induced stress on animals and plants are relatively well known. For example, in humans, microgravity conditions result in bone loss, upwards to 3% per month (Lynch and Matin, 2005; Globus and Morey-Holton, 2009; Stein, 2013), permutations to both the adaptive and innate immune systems (Guéguinou *et al.*, 2009), and an increased potential risk of bacterial and viral infections (Ott *et al.*, 2012; Mermel, 2013).

Conclusion

This research has been able to identify and prove the capability of some microorganisms to survive in the presence of preservative in cosmetics used. Microbiological safety is one of the most dynamic and critical of cosmetics quality parameters. The most frequently isolated bacteria namely *Staphylococcus aureus* exhibited greater resistance to antibiotics under stimulated microgravity condition than one under earth gravity condition at different time.

References

Allen, C. A., Niesel, D. W. and Torres, A. G. (2008). The effects of low-shear stress on Adherent invasive *Escherichia coli. Environmental Microbiology*, 10: 1512-1525.

Audia, J. P., Webb, C. C. and Foster, J. W. (2001). Breaking through the acidbarrier: an orchestrated response to proton stress by enteric bacteria. *International Journal of Medical Microbiology*,291: 97–106.

Barnett, H. L. and Hunter, B. B. (1998). Illustrated genera of imperfect fungi. 4th edition. St. Paul, Minn: APS Press

Bauer, A.W., Kirby, W. M., Sherris, J. C. and Turck, M. (1996). Antibiotic susceptibility testing by a standardized single disk method. *American journal of Clinical Pathology*,459(4): 493-6.

Brown, R. B., Klaus, D. and Todd, P. (2002). Effects of space flight, clinorotation, and centrifugation on the substrate utilization efficiency of *E. coli. Microgravity Science and Technology*, 13: 24-29.

Cavicchioli, R., Thomas, T. and Curmi, P. M. (2000). Cold stress response in Archaea. *Extremophiles*, 4:321–331.

Cheesbrough, M. (2010). *District Laboratory Practice in Tropical Countries*, 2nd edition, Cambridge University press, Cambridge University Press, New York, pp. 70 -71.

Demain, A. L. and Fang, A. (2001). Secondary metabolism in simulated microgravity. *Chemical Record*, 1: 333-346.

Dickson, K. J. (1991). Summary of biological spaceflight experiments with cells. *ASGSB Bull*, 4:151–260.

Food and Drug Administration. (1992). *Cosmetic handbook-US. Center for Food safety and applied nutrition*. FDA/IAS-Booklet

Foster, J. W. and Spector, M. P. (1995). How Salmonella survive against the odds. *Annual Review Microbiology*, 49: 145–174.

Globus, R. K. and Morey-Holton, E. R. (2009). Advances in understanding the skeletal biology of spaceflight. *Gravitational and Space Biology*, 22: 3–12

Guéguinou, N., Huin-Schohn, C., Bascove, M., Bueb, J. L., Tschirhart, E., Legrand-Frossi, C. and Frippiat, J. P. (2009). Could spaceflight-associated immune system weakening preclude the expansion of human presence beyond Earth's orbit? *Journal of Leukocyte Biology*, 86: 1027–1038.

Hecker, M. and Volker, U. (2001). General stress response of *Bacillus subtilis* and other bacteria. *Advances in Microbial Physiology*, 44: 35–91.

Hengge-Aronis, R. (2002). Recent insights into the general stress response regulatory network in *Escherichia coli*. *Journal of Molecular Microbiology and Biotechnology*, 4: 341–346.

Hitchins, A. D., Tran, T. T. and McCarron, J. E. (2001). *Microbiological Methods for Cosmetics*; Chapter 23. Bacteriological Analytical Manual. 8th Edition, Revision A, 1998.

Kacena, M. A., Merrell, G. A., Manfredi, B., Smith, E. E., Klaus, D. M. and Todd, P. (1999). Bacterial growth in spaceflight: logistic growth curve parameters for *Escherichia coli* and *Bacillus subtilis*. *Applied Microbiology* and *Biotechnology*, 51: 229–234.

Klaus, D. M. (2002). *Space microbiology: microgravity and microorganisms*. p. 2996–3004.InG. Bitton (ed.), Encyclopedia of environmental microbiology. John Wiley & Sons, Inc., New York, N.Y.

Lundov, M. D., Moesby, L., Zachariae, C. and Johansen, J. D. (2009). Contamination versus cosmetics: A review on legislation, usage, infections and contact allergy. *Contact Dermatitis*,60: 70-78

Lynch, S . V., Brodie, E. L. and Matin, A. (2004). Role and regulation of sigma S in general resistance conferred by low-shear simulated microgravity in *Escherichia coli*. *Journal of Bacteriology*, 186: 8207-8212.

Lynch, S. V. and Matin, A. (2005). Travails of microgravity: Man and microbes in space. *Biologist*, 52(2): 80–87.

Matin, A., Lynch, S. V. and Benoit, M. R. (2006). Increased bacterial resistance and virulence in simulated microgravity and its molecular basis. *Gravitational and Space Biology*, 19(2): 31-41.

Mauclaire, L. and Egli, M. (2010). Effect of simulated microgravity on growth and production of exopolymeric substances of *Micrococcus luteus* space and earth isolates. *FEMS Immunology and Medical Microbiology*,59: 350-356.

McLean, R. J., Cassanto, J. M., Barnes, M. B. and Koo, J. H. (2001). Bacterial biofilm formation under microgravity conditions. *FEMS Microbiology Letters*, 195: 115-119.

Mermel, L. A. (2013). Infection prevention and control during prolonged human space travel. *Clinical Infection*, 56: 123–130.

Mishra, S. K. and Pierson, D. L. (1992). *Spaceflight: effects on microorganisms*. pp. 53–60. In J. Lederberg (ed.), Encyclopedia of microbiology, vol. 4. Academic Press, Inc., San Diego, Calif.

Mwambete, K. D. and Simon, A. (2010). Microbiological quality and preservative capacity of commonly available cosmetics in Dares Salaam, Tanzania. *East and Central African Journal of Pharmaceutical Sciences*,13: 5

Nickerson, C. A., Ott, C. M., Mister, S. J., Morrow, B. J., Burns-Keliher, L. and Pierson, D. L. (2000). Microgravity as a novel environmental signal affecting *Salmonella entericsero var typhimurium* virulence. *Infection and Immunity*, **68**: 3147-3152.

Nickerson, C. A., Ott, C. M., Wilson, J. W., Ramamurthy, R., LeBlanc, C. L., Honerzu Bentrup, K., Hammond, T. and Pierson, D. L. (2003). Low-shear modeled microgravity: a global environmental regulatory signal affecting bacterial gene expression, physiology, and pathogenesis. *Journal of Microbiology Methods*, 54: 1–11.

Olutiola, P.O., Famurewa, O. and Senntag, H.G. (2000). *An introduction to General microbiology*, Hygiene institute Der Universital Heideberg. Federal Republic of Germany. pp 267.

Ott, C. M., Crabbe, A., Wilson, J. W., Barrila, J., Castro, S. L. and Nickerson, C. A. (2012). *Microbial Stress: Spaceflight-Induced Alterations in Microbial Virulence and Infectious Disease Risks for the Crew*. In Stress Challenges and immunity in Space; Chouker, A., Ed.; Springer-Verlag: Berlin/Heidelberg, Gemany, 2012; pp. 203–225.

Oyeleke, S. B., Dauda, B. E. N. and Boye, O. A. (2008). Antibacterial activity of *Ficuscapensis*. *African Journal of Biotechnology*, 7(10): 1414-1417.

Poolman, B., Blount, P., Folgering, J. H., Friesen, R. H., Moe, P. C. and Heide, T. V. (2002). How do membrane proteins sense water stress? *Molecular Microbiology*, 44:889–902.

Rosenzweig, J. A., Abogunde, O., Thomas, K, Lawal, A., Nguyen, Y., Sodipe, A. and Jejelo, O. (2010). Spaceflight and modeled microgravity effects on microbial growth and virulence. *Applied Microbiology and Biotechnology*, 85: 885–891.

Stein, T. P. (2013). Weight, muscle and bone loss during space flight: Another perspective. *European Journal of Applied Physiology*, 113: 2171–2181.

The European Cosmetic Toiletry and Perfumery Association. (1994). *Cosmetic Good Manufacturing Practices, COLIPA*.

Wilson, J. W., Ott, C. M, Bentrup, Z., Ramamurthy, R., Quick, L., Porwollik, S., Cheng, P., McClelland, M., Tsaprailis, G., Radabaugh, T., Hunt, A., Fernandez, D., Richter, E., Shah, M., Kilcoyne, M., Joshi, L., Nelman-Gonzalez, M., Hing, S., Parra, M., Dumars, P., Norwood, K., Bober, R., Devich, J., Ruggles, A., Goulart, C., Rupert, M., Stodieck, L., Stafford, P., Catella, L., Schurr, M. J., Buchanan, K., Morici, L., McCracken, J., Allen, P., Baker-Coleman, C., Hammond, T., Vogel, J., Nelson, R., Pierson, D. L., Stefanyshyn-Piper, H. M. and Nickerson, C. A. (2007). Space flight alters bacterial gene expression and virulence and reveals a role for global regulator Hfq. *Proceedings of National Academy of Sciences USA*, 104: 16299-16304.