

Antibiotic susceptibility profile of bacilli isolated from the skin of healthy humans

Prashant Tarale, Sonali Gawande, Vinay Jambhulkar

Department of Biotechnology, Sindhu Mahavidyalaya Center for Biotechnology, Maharashtra, India.

Submitted: December 30, 2013; Approved: October 7, 2014.

Abstract

In the present work, twelve bacilli were isolated from four different regions of human skin from Bela population of Nagpur district, India. The isolated bacilli were identified by their morphological, cultural and biochemical characteristics. Seven isolates were Gram negative rods, out of which five were belong to genus *Pseudomonas*. Three among the five Gram positive isolates were identified as *Dermabactor* and the remaining two *Bacillus*. Their antimicrobial susceptibility profile was determined by Kirby-Bauer disc diffusion method. The isolates showed resistance to several currently used broad-spectrum antibiotics. The *Dermabactor* genus was resistant to vancomycin, although it was earlier reported to be susceptible. Imipenem was found to be the most effective antibiotic for *Pseudomonas* while nalidixic acid, ampicillin and tetracycline were ineffective. Isolates of *Bacillus* displayed resistance to the extended spectrum antibiotics cephalosporin and ceftazidime. Imipenem, carbenicillin and ticarcillin were found to be the most effective antibiotics as all the investigated isolates were susceptible to them. Antibiotic resistance may be due to the overuse or misuse of antibiotics during the treatment, or following constant exposure to antibiotic-containing cosmetic formulations.

Key words: antibiotic resistance, identification, microflora, nosocomial, opportunistic.

Introduction

Human skin is considered to be a critical barrier between the human body and its outer environment. It prevents loss of moisture and restricts the entry of pathogenic organism (Bojar and Holland, 2002). Hence it seems to be an excellent ecosystem that harbors varying microbial communities (Kong and Segre, 2012). These microbial communities are distributed across the human skin and live in physiologically diverse and topographically distinct niches, depending on the environmental conditions specific to distinct region of the skin (Grice *et al.*, 2009; Oh *et al.*, 2012). The normal flora generally coexists asymptotically with host, but can cause infection whenever the host immune system gets compromised or skin is damaged. Microflora causing such infection are said to be opportunistic pathogens.

The contribution of Gram negative bacilli as normal microflora of the human skin is quite small as compared to their extraordinary numbers in the human gut (Lockhart *et al.*, 2007). This may be attributed to desiccation considered

a major factor preventing the colonization and multiplication of Gram negative organisms on skin (Elsner, 2006). Gram negative organisms such as *Pseudomonas aeruginosa*, *Pasteurella multocida*, *Vibrio vulnificus* are not considered typical resident of human skin microflora since they may cause cutaneous infections (Larson *et al.*, 1986a, 1986b).

Bacteria can infect human skin when they gain access to the human host. This happens when the host has the broken skin or mucus membrane or is immunocompromised (Hogenova *et al.*, 2004). The treatment of infections then depends on use of different antibiotics treatments. It is plausible that this may account for phenomenon of emergence of resistance of bacteria to various antimicrobial agents (Patil and Chopade, 2001). The development of antimicrobial resistance reflects an evolutionary process in response to the antimicrobial therapy that may select physiologically or genetically competent strain capable of surviving high dose of antimicrobial agents (Zhang Li and Liu, 2007). The acquired resistance may be transfer in the

form of resistance genes to other species via horizontal gene transfer (Pardesi *et al.*, 2007; Barak *et al.*, 2005). In the recent times, information is increasing on cosmetic products contain antibiotics may again drive the development of antibiotic resistance among the normal microflora of human skin (Hegstad *et al.*, 2010). In a related study antibiotic resistant strains of human microflora were isolated from peoples who did not have prior contact with hospital environment, and from doctors who were closely connected with hospital. It was shown that isolated strain from the doctors, who were not under any kind of antibiotic treatment, were five times more multidrug resistant to antibiotics used in hospital environment (Kwaszewska *et al.*, 2009; Berlau *et al.*, 1999).

Although, studies have reported the role of skin bacilli in nosocomial infections, but bacilli are still underexplored with respect to their antibiotic resistance profile. Antibiotic-resistant flora from human skin can potentially transmit the antibiotic resistance gene pool to other known pathogens of human skin. Hence, the need for routine examination of antibiotic susceptibility of isolates from human skin is important. In this study, isolation of pure culture of bacilli from human skin samples was carried out. The isolated bacilli were identified using morphological, cultural and biochemical characteristics, after which antibiotic resistance profile studied, to gain better understanding about the antibiotic resistance of skin microflora. The finding from this is expected to guide definitive antibiotic therapy.

Material and Methods

Sample collection from human skin

Samples were collected from twelve healthy people of Bela village from Nagpur district. The people were within the the age group of 18 to 45 years and with no observable symptomatic disease. The use of cosmetics was not considered as selection criteria as the study population was from a village and used very little or no cosmetics. The samples were collected by rubbing sterile cotton swab on skin and transferred to tube containing nutrient broth. Samples were collected from four different regions of healthy human skin, making a total of twelve samples (Table 1). Moist regions of the skin that favor the growth and colonization of bacteria were selected for sample collection. Each

sample was coded by a number and an alphabet corresponding to the sites selected.

Identification of bacilli from collected samples

Isolation of pure culture from the samples was carried out by streak plate method. The isolates were identified by their morphological, cultural and biochemical characteristics. The pure cultures were characterized using different methods of microscopic observation including Gram staining, motility. The cultural characteristics on solid media include observation of colony size, margin, surface, color, elevation. Nutrient agar was used for the study. The biochemical characterization include sugar test, IMViC, and demonstration of enzyme activity like starch hydrolysis, tributyrin hydrolysis, oxidase, catalase, nitrate reduction and urease.

Antibiotic susceptibility testing

The antibiotics used along with their abbreviations (as per CLSI) and concentration are mentioned as follows; Amoxicillin AMR (30 µg/disc), Ampicillin AMP (25 µg/disc), Carbenicillin CAR (100 µg/disc), Ceftazidime CAZ (30 µg/disc), Cephalothin CEF (30 µg/disc), Ciprofloxacin CIP (30 µg/disc), Cloxacillin CLX (30 µg/disc), Gentamycin GEN (30 µg/disc), Imipenem IPM (10 µg/disc), Kanamycin KAN (30 µg/disc), Methicillin MET (30 µg/disc), Nalidixic acid NAL (30 µg/disc), Piperacillin PIP (100 µg/disc), Roxithromycin ROX (30 µg/disc), Streptomycin STR (10 µg/disc), Tetracycline TET (30 µg/disc), Ticarcillin TIC (30 µg/disc), Tobramycin TOB (30 µg/disc), Vancomycin VAN (100 µg/disc). The antibiotic discs (6 mm diameter were purchased from Hi-media Laboratory Ltd., Mumbai (India). The Kirby-Bauer method of antibiotic sensitivity testing is a relatively simple, reliable and rapid test was used in this study. The effectiveness of antimicrobial in sensitivity testing is based on the size of zone of inhibition that surrounds a disc that has been impregnated with a specific concentration of an agent. The Kirby-Bauer method is a standardized system for antimicrobial resistance profiling. Plates were prepared with Mueller Hinton agar. Inoculation was carried out by spread plate method. Isolated bacilli were grown in nutrient broth up to 1.0 OD and 100 µL of each was spread uniformly by spreader on agar surface. Various antibiotic discs then placed on the agar plate and the plates were incubated at 37 °C for 24 h. After 24 h incubation, plates were observed for zone of inhibition around the antibiotic discs. The zone of inhibition for different antibiotics was then measured. The antibiotics used for the antibiotic resistance profiling were selected on the basis of their class as described along with their abbreviation and concentration (µg/disc). The spectrum of activity was also considered for selection of antibiotics. For example, broad spectrum tetracyclines, quinolones and third generation

Table 1 - Regions of skin from different people depicting the name of sample.

Skin Region	Sample
Inner Elbow	9IE, 4IE, 15IE, 10IE, 7IE2
Philtrum	10J, 7J, 14J, 12JY
Cheek	5CT, 14C
Forehead	14FH

cephalosporins are active against both Gram negative and Gram positive organisms, while narrow spectrum antibacterial drugs have limited activity.

Amplification of 16S rRNA and lipase A genes

Nutrient agar is generally used for bacterial growth but can also support the growth of many microorganisms other than bacteria which can confound the outcome of a result. In order to eliminate this possibility, PCR amplification with eubacteria-specific 16S rRNA universal primers was carried out. Since these primers are known to be highly specific for eubacteria, amplification with these primers could confirm the isolates as bacteria. Isolation of genomic DNA was carried out from all the isolates. Cell lysis occurs due to the action of SDS (Sodium dodecyl sulphate) which destabilizes the plasma membrane. Upon centrifugation, cell debris along with trapped RNA, proteins are separated. Resulting supernatant contains genomic DNA. This was followed by the amplification of 16S rRNA gene with universal primers (FP Bac AGAGTTTTATCCTGGTCAG, RP univ592r ACCGCGGCKGCTGGC). 1.5 μ L of template DNA was used for amplification of genomic DNA for 40 cycles (denaturation 94 °C-10 min; denaturation, 94 °C-1 min; annealing 54.9 °C- 1 min; polymerization 72 °C-10 min). Amplification was also carried out for Lipase A gene to validate the results obtained on tributyrin agar plates for lipid hydrolysis (Figure 1) (Lindh *et al.*, 2005). 1.5 μ L of genomic DNA was used for amplification with Lipase A primers (FP L1 ATGGTTCACGGTATTGGA GG, RP L2 CTGCTGTAAATGGATGGAGG) for 40 cycle with condition of denaturation 94 °C-10 min; denaturation, 94 °C- 1 min; annealing 54.6 °C- 1 min; polymerization 72 °C- 10 min. The PCR product length for 16S rRNA and Lipase A gene was 540 bp and 371 bp respectively.

Results

Isolation and identification of bacilli from skin samples

The pure cultures of bacteria from 12 skin samples were obtained by streak plate method. The isolated pure cultures were then identified based on their morphological, cultural and a biochemical characteristic with reference to Bergey's manual of determinative bacteriology (Brockman, 1986). The results are summarized in Table 2. From twelve isolated pure cultures, seven were found to be Gram negative while others were Gram positive. The characteristics observed were then compared with Bergey's manual of determinative bacteriology and the isolates were categorized to genus level and to species level wherever possible.

Antibiotic resistance profile of identified bacilli

After identification, the antibiotic susceptibility of all isolates was determined. The results for the antibiotic susceptibility are shown in Table 3 and 4. The seven Gram

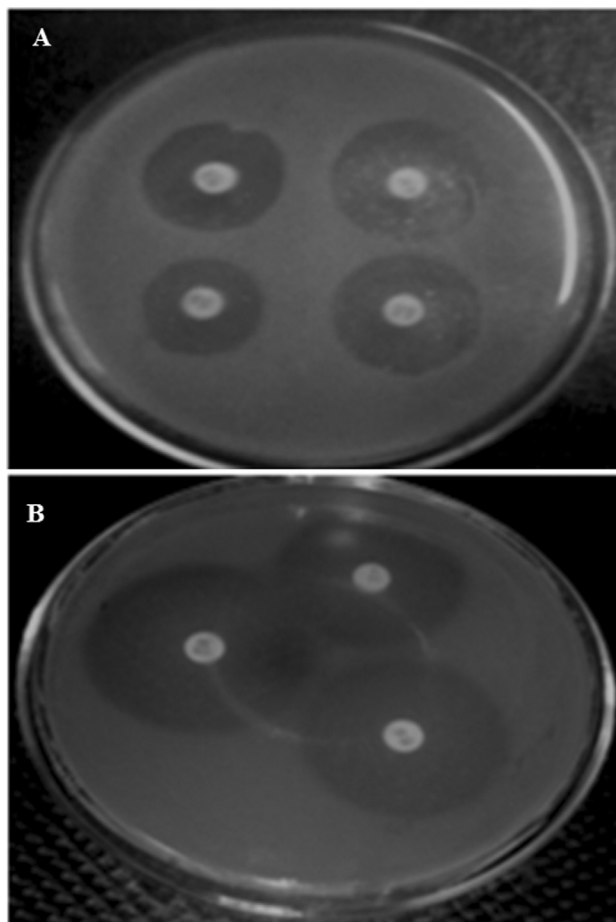


Figure 1 - Representative result for antibiotic susceptibility profile. Zone of inhibition obtained on Mueller Hinton (MH) agar for (A) 10IE isolate and (B) 7IE2 isolate.

negative isolates were tested for 19 antibiotics. They were found to be resistant to streptomycin, nalidixic acid and ampicillin, while three of the isolates were resistant to cephalothin and tetracycline. It was also found that Imipenem, carbenicillin and ticarcillin were the most effective antibiotic to which all Gram negative isolates were susceptible. The five Gram positive isolates were found to be highly resistant to ceftazidime antibiotic. Two isolates were also resistant to cephalothin and vancomycin, while all Gram positive isolates were susceptible to Imipenem and methicillin.

Six isolates from inner elbow region of different persons were found to be resistant to nalidixic acid, ampicillin and tetracycline. Out of which three isolates were highly resistant to cephalothin. Among the 19 antibiotics Imipenem, ticarcillin, carbenicillin and piperacillin were the most effective to which all isolate were susceptible. Four isolates from philtrum region were found to be highly resistant to ceftazidime and two of them were also resistant to nalidixic acid and vancomycin while the other were also resistant to streptomycin, tobramycin and piperacillin. All isolates showed susceptibility to Imipenem, carbenicillin and ticar-

Table 2 - Identification chart for the isolates based on the Bergey's manual of Determinative Bacteriology.

Characteristics	<i>P. stutzeri</i> 9IE	<i>P. mendocina</i> 4IE	<i>P. stutzeri</i> 15IE	<i>P. mendocina</i> 14FH	<i>P. mendocina</i> 14C	<i>Aeromonas</i> spp. 14J	<i>Proteus</i> spp. 7J	<i>B. licheniformis</i> 5CT	<i>Sulfobacillus</i> 10IE	<i>Dermabactor</i> spp. 10J	<i>Dermabactor</i> spp. 12JY	<i>Dermabactor</i> spp. 7IE2
Gram staining	-	-	-	-	-	-	-	+	+	+	+	+
Endospore								+	+			
Motility	+	+	+	+	+	+	+	+	-	-	-	-
Catalyse	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	+	-	+	+	+	+	+	+	+	-	-	-
Glucose fermentation	+	+	+	-	-	-	-	+	+	+	+	-
Lactose fermentation	-	-	-	-	-	-	-	-	+	-	-	-
Mannitol fermentation	-	-	-	-	-	-	-	+	+	+	-	-
IMViC I	-	-	-	-	-	-	-	-	-	-	-	-
M	-	-	-	-	-	-	-	-	-	-	-	-
VP	-	-	-	-	-	-	-	-	-	-	-	-
C	+	-	+	-	-	-	-	+	-	+	-	-
Starch Agar	+	-	+	-	-	+	-	+	+	-	-	-
Tributyrin Agar	+	-	+	+	+	+	-	+	-	+	-	-
Urease	-	-	-	+	+	-	+	+	+	-	-	+
Nitrate reduction	+	+	+	-	-	+	-	+	+	-	-	-

+ Positive result; - Negative result.

cillin. Figure 2 shows the zone of inhibition obtained around antibiotic disc for two isolates 10IE and 7IE2 after 24 h of incubation.

Amplification of 16S rRNA and Lipase A genes

Microorganism other than bacteria, like yeasts, may form colonies on nutrient agar. Hence, PCR amplification

Table 3 - Antimicrobial susceptibility pattern for Gram negative isolates.

Antibiotics	Susceptibility of isolates to antibiotics with diameter of inhibition (mm) shown in bracket						
	<i>P. stutzeri</i> (9IE)	<i>P. luteolla</i>	<i>P. stutzeri</i>	<i>Proteus</i> spp.	<i>Aeromonas</i> spp.	<i>P. mendocina</i>	<i>P. mendocina</i>
GEN	R(16)	S(22)	S(20)	I(17)	I(18)	I(16)	I(15)
KAN	R(13)	I(16)	S(22)	S(18)	S(18)	S(18)	S(18)
TOB	S(21)	R(16)	I(18)	I(16)	I(16)	I(15)	I(16)
STR	S(11)	R(15)	S(21)	S(22)	S(18)	R(00)	R(00)
NAL	R(11)	R(00)	I(14)	R(09)	I(18)	R(06)	R(08)
CIP	R(24)	S(28)	S(34)	I(28)	S(33)	R(08)	I(18)
IPM	S(36)	S(32)	S(30)	S(29)	S(32)	S(28)	S(26)
PIP	S(20)	S(22)	S(20)	S(35)	R(16)	S(18)	S(18)
TIC	S(24)	S(22)	S(22)	S(22)	S(19)	S(28)	S(35)
CAR	S(24)	S(28)	S(21)	S(15)	S(21)	S(30)	S(28)
VAN	R(00)	I(22)	S(22)	S(22)	R(16)	S(18)	S(19)
AMP	R(07)	R(11)	R(18)	S(21)	R(09)	R(25)	R(18)
MET	S(22)	R(15)	I(20)	S(28)	S(25)	R(15)	S(30)
CAZ	S(24)	S(22)	S(22)	R(08)	R(00)	I(12)	S(20)
CEF	R(00)	S(30)	R(00)	S(20)	R(00)	S(22)	S(21)
TET	R(16)	R(17)	R(15)	S(19)	I(15)	I(28)	S(21)

R- Resistance, S- Susceptible, I- Intermediate sensitivity.

Table 4 - Antimicrobial susceptibility pattern for Gram positive isolates.

Antibiotics	Susceptibility of isolates to antibiotics with zone of inhibition (mm) shown in bracket				
	<i>Sulfobacillus</i>	<i>Dermabactor</i> spp.	<i>Dermabactor</i> spp.	<i>Dermabactor</i> spp.	<i>B. licheniformis</i>
GEN	S(19)	R(12)	R(13)	I(14)	S(20)
KAN	S(19)	I(16)	S(20)	R(08)	S(20)
TOB	I(16)	R(15)	I(16)	R(10)	S(18)
STR	S(20)	S(15)	S(22)	R(00)	S(18)
NAL	I(16)	I(15)	R(00)	R(11)	I(16)
CIP	S(35)	S(31)	R(08)	I(18)	S(33)
IPM	S(40)	S(37)	S(30)	S(40)	S(40)
PIB	S(24)	R(17)	S(21)	S(27)	R(11)
TIC	S(25)	S(19)	S(28)	S(40)	R(12)
CAR	S(22)	S(20)	S(32)	S(40)	R(09)
VAN	S(19)	R(13)	R(09)	S(20)	S(17)
TET	S(27)	R(16)	S(26)	S(30)	R(15)
MET	S(22)	I(19)	S(29)	S(29)	I(15)
CLX	I(20)	I(18)	S(31)	S(30)	R(06)
CAZ	S(19)	R(00)	R(00)	R(00)	R(10)
ROX	R(13)	S(33)	S(26)	I(17)	R(00)
CEF	S(27)	S(38)	R(00)	S(22)	R(00)
TET	S(26)	S(28)	R(13)	S(30)	I(18)

R- Resistance, S- Susceptible, I- Intermediate sensitivity.

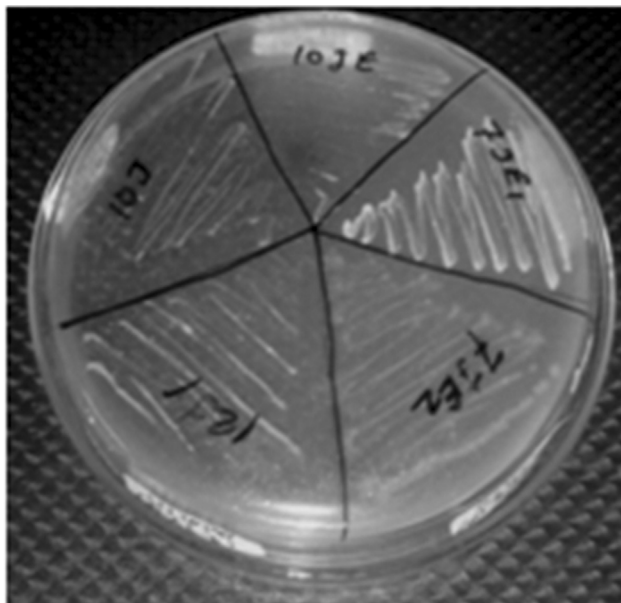


Figure 2 - Representative result for lipid hydrolysis on tributyrin agar.

with eubacterial 16S rRNA-specific primers was carried out, as 16S rRNA gene is highly conserved across the bacterial kingdom. The 16S rRNA gene of all isolates was partially amplified with 16S rRNA universal primers and PCR products (540 bp) obtained were run on 1.2% agarose gel. Amplification of the gene for 16s rRNA was successfully carried out for confirming that the isolates were bacterial species (Figure 3). Study of lipid hydrolysis is included in the biochemical characterization of isolates. Lipid hydrolysis was studied on tributyrin agar. Seven isolates were found positive for lipid hydrolysis (Figure 1). Lipase A primers was used for validating the result obtained for lipid hydrolysis on tributyrin agar. Lipid hydrolysis is a significant virulence factor for skin pathogenesis. Results of amplification with Lipase A primers confirmed the presence of this virulence factor and validate the results obtained on tributyrin agar for lipid hydrolysis as shown in Figure 4.

Discussion

An enhanced understanding of the skin microbiome is necessary to gain insight into microbial involvement in human skin disorder, and to enable novel promicrobial and antimicrobial therapeutic approaches for their treatment. Hence it is important to know the antibiotic susceptibility of isolates from human skin (Todar, 2012; Wilson, 2008). The significance of coryneforms in opportunistic infections is growing especially in immunocompromised patients. Coryneforms have been described as the main etiologic factors of opportunistic infections (Kazmierczak *et al.*, 2005). For instance Dermabactor is a relatively new genus and *D. hominis* is a relatively new species. *D. hominis* has been assigned in to Coryneform group 3 and group 5 respectively. Little has been learnt about its epidemiology except that it is found among the human cutaneous flora (Funke *et al.*, 1996; Kazmierczak *et al.*, 2005). In previous studies Dermabactor was found to be resistant to aminoglycoside, fluoroquinolones, macrolides and lincosamide. In present work three isolates of Dermabactor displayed high resistance to ceftazidime, and two of them also showed resistance to aminoglycoside (tobramycin) and quinolones (nalidixic acid) (Funke *et al.*, 1996). Dermabactor, which was reported to be susceptible to glycopeptides (vancomycin) earlier, displayed resistance to vancomycin in this study. In another study Southern African strain of *B. anthracis* was studied for its susceptibility to newly developed antibiotics (Frean *et al.*, 2003). In previous study lack of activity of extended spectrum cephalosporin against *B. anthracis* was reported (Paavilainen *et al.*, 2000). In this study one isolate of Bacillus displayed resistance to cephalosporin and ceftazidime. In another prime study, *Pseudomonas stutzeri* shown susceptibility to nalidixic acid and resistance to ampicillin and streptomycin (Hentges *et al.*, 1985; Lalucat *et al.*, 2006). In present work isolates of *Pseudomonas* displayed resistance to nalidixic acid, ampicillin and streptomycin, while they showed susceptibility to ceftazidime, piperacillin and ticarcillin. Imipenem was

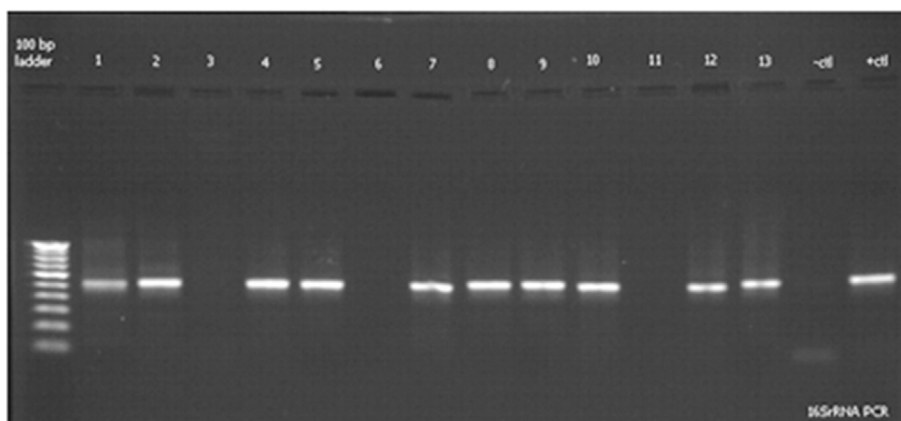


Figure 3 - Agarose gel showing amplification of 16S rRNA genes from different isolates. Lane 1- 9IE, 2- 4IE, 3- 15IE, 4- 7IE2, 5- 7J, 6- 10IE, 7- 10J, 8- 14J, 9- 14FH, 10- 14C, 11- 12JY, 12- 13C, 13- 5CT.

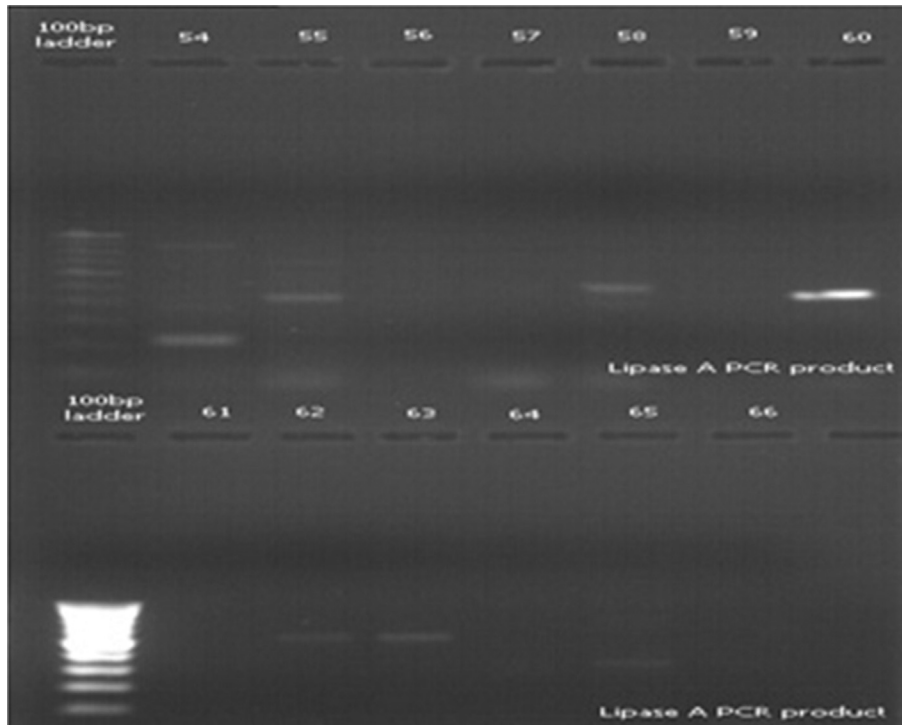


Figure 4 - Agarose gel amplification of Lipase A gene from different isolates. Lane 54- 9IE, 55- 4IE, 56- 15IE, 57- 7IE2, 58- 7J, 59- 10IE, 60- 10J, 61- 14J, 62- 14FH, 63- 14C, 64- 12JY, 65- 13C, 66- 5CT.

found to be the most effective antibacterial for *Pseudomonas* in agreement with previous studies.

On comparing the results from the present study with previous studies, it can be considered that the antibiotic resistance has increased in skin isolates. Antibiotics to which isolates were found susceptible in previous study were ineffective in present work. Isolates from skin of healthy humans showed resistant to board spectrum of antibiotics. Skin colonizing normal microbial flora generally resides peacefully without harming host, but may cause infection upon physical injury or in case of immunocompromised hosts. The presence of high antimicrobial resistance in skin microbiome can be a cause for concern as there is probability of horizontal gene transfer of the antibiotic resistance gene pool to skin pathogens. This can become a serious challenge in clinical therapy. It can be concluded that this increasing antibiotic resistance may be a result of uncontrolled utilization of antibiotics. Therefore skin of healthy human can be considered to be one of the most important reservoirs for microorganism causing clinically acquired infections. The study has reported that cosmetic formulations also contain antibiotics and drive antibiotic resistance in skin microflora (Horner *et al.*, 2012). The use of cosmetics even though not considered for selecting individuals in present work but still, it may be considered as a contributing factor for development of antibiotic resistance in skin microflora. Untreated wastewater from antibiotic industry may aid in developing the reservoir of antibiotic resistance gene pool in environmental bacteria. These resistance

genes may be transfer to human microbiome including pathogens (Cabello *et al.*, 2013; Li *et al.*, 2010). In addition to this, detergents like quaternary ammonium salt can also be responsible for development of cross resistance against antibiotics (Hegstad *et al.*, 2010).

References

- Barak O, Treat JR, James WD (2005) Antimicrobial peptides: Effect of innate immunity in the skin. *Adv Dermatol* 2:357-374.
- Berlau J, Aucken H, Malnick H *et al.* (1999) Distribution of *Acinetobacter* species of skin of healthy human. *Eur J Clin Microbiol Infect* 18:179-183.
- Bojar KA, Holland KT (2002) The human cutaneous microbiota and factors controlling colonization. *World J Microbiol Biotechnol* 18:889-903.
- Brockman ER (1986) *Bergey's Manual of Systematic Bacteriology*. Volume 3. Edited by Sneath PHA, Mair NS, Sharpe ME, Holt JG. Williams & Wilkins, Baltimore.
- Cabello F, Godfrey H, Tomova A *et al.* (2013) Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health. *Environ Microbiol* 15:1917-1942.
- Elsner P (2006) Antimicrobial and the skin physiological and pathological flora. *Dermatol* 33:35-41.
- Frean J, Klugman KP, Arntzen L *et al.* (2003) Susceptibility of bacillus to eleven antimicrobial agents including novel fluoroquinolones and ketolides. *J Antimicrob Chemother* 52:297-299.
- Funke G, Verena PU, Graevenitz AV (1996) Antimicrobial Susceptibility Patterns of Some Recently Established Cory-

- neform Bacteria. *Antimicrob Agents Chemoter* 40:2874-2878.
- Grice EA, Kong HH, Conlan S *et al.* (2009) Topographical and temporal diversity of the human skin microbiome. *Science* 324:593-595.
- Hegstad K, Langsrud S, Lunestad BT *et al.* (2010) Does the wide use of quaternary ammonium compounds enhance the selection and spread of antibacterial resistance and thus threaten our health. *Microbiol Drug Resist* 16:91-104.
- Hentges DJ, Stein AJ, Casey SW *et al.* (1985) Protective role of intestinal flora against *Pseudomonas aeruginosa* in mice: influence of antibiotics on colonization resistance. *Infect Immun* 47:118-122: 2856912.
- Hogenova HT, Stepankova R, Hudcovic T *et al.* (2004) Commensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases. *Immunol Lett* 93:97-108.
- Horner C, Mawer D, Wilcox M (2012) Reduced susceptibility to chlorhexidine in staphylococci: is it increasing and does it matter? *J Antimicrob Chemother* 67:2547-2559.
- Kazmierczak AK, Kwaszewska JK, Szewczyk EM (2005) Opportunistic Coryneform Organism- Resident of Human Skin. *Pol J Micriobiol* 54:27-37.
- Kazmierczak AK, Szewczyk EM (2004) Bacteria forming a resident flora of the skin as a potential source of opportunistic infections. *Pol J Micriobiol* 53:249-255.
- Kenneth Todar (2012) *Online Textbook of Bacteriology*. University of Wisconsin, Department of Bacteriology.
- Kong HH, Segre JA (2012) Skin Microbiome Looking Back to move forward. *J Invest Dermatol* 132:933-939.
- Kwaszewska AK, Sobis-Glinkowska M, Szewczyk EM (2009) Influence of contact with hospital environment on sensitivity to antibiotic to lipophilic strains of *Corynebacterium* residing on human skin. *Med Dosw Mikrobiol* 61:359-366.
- Lalucat J, Bennisar A, Bosch R *et al.* (2006) Biology of *Pseudomonas stutzeri*. *Microbiol Mol Biol Rev* 70:510-547.
- Larson EL, McGinley KJ, Foglia AR *et al.* (1986) Composition and Antimicrobial Resistance of Skin Flora in Hospitalized and Healthy Adults. *J Clin Microbiol* 23:604-608.
- Larson EL, McGinley KJ, Foglia AR *et al.* (1986) Skin colonization with antibiotic-Resistance (JK Group) and Antibiotic-Sensitive Lipophilic diptheroids in Hospitalized and Normal Adults. *Jpn J Infect Dis* 153:701-706.
- Li D, Yu T, Zhang Y *et al.* (2010) Antibiotic Resistance Characteristics of Environmental Bacteria from an Oxytetracycline Production Wastewater Treatment Plant and the Receiving River. *Appl Environ Microbiol* 76:3444-3451.
- Li M, Zhang GA, Liu Y (2007) Analysis of predominant bacteria from burn infections and their resistance to antibiotics in recent years. *Chin J Burns* 23:255-259.
- Lindh JM, Terenius O, Faye I (2005) 16S rRNA gene-based identification of midgut bacteria from field-caught *Anopheles gambiae* sensu lato and *A. funestus* mosquitoes reveals new species related to known insect symbionts. *Appl Environ Microbiol* 71:7217-7223.
- Lockhart SR, Abramson MA, Beekmann SE *et al.* (2007) Antimicrobial resistance among Gram negative bacilli causing infection in intensive care unit in United State between 1993 and 2004. *J Clin Microbiol* 45:3352-3359.
- Oh J, Conlan S, Polley EC *et al.* (2012) Shift in human skin and nares microbiota of healthy children and adults. *Genome Med* 4:1-11.
- Paavilainen T, Osterblad M, Leistevuo T *et al.* (2000) Screening for antimicrobial resistance in normal bacterial flora of the skin using the replica plating method. *Eur J Clin Microbiol Infect* 21: 956-959.
- Pardesi KR, Yavankar SP, Chopade BA (2007) Plasmid distribution and antimicrobial susceptibility of *Acinetobacter* genospecies from healthy tribal population of western India. *Indian J Med Res* 125:79-88.
- Patil JR, Chopade BA (2001) Distribution and in vitro antimicrobial susceptibility of *Acinetobacter* species on the skin of healthy humans. *Natl Med J India* 14:204-208.
- Wilson M (2008) *Bacteriology of Humans: An Ecological Perspective*. Wiley-Blackwell, Hoboken, 360 p.

Associate Editor: Roxane Maria Fontes Piazza

All the content of the journal, except where otherwise noted, is licensed under a Creative Commons License CC BY-NC.