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SPECIES DISTRIBUTION AND PHYSIOLOGICAL CHARACTERIZATION OF ACINETOBACTER GENOSPECIES FROM HEALTHY HUMAN SKIN OF TRIBAL POPULATION IN INDIA

SP Yavankar, KR Pardesi, *BA Chopade

Abstract

Background: Various reports on distribution of Acinetobacter spp. from healthy human skin restricted to urban population. However, no such data is available from healthy human skin of tribal population not exposed to modern antibiotics during their life time. Purpose: Isolation, biotyping, distribution and physiological characterisation of Acinetobacter spp. from healthy human skin of tribal population. Methods: Tribal population of Toranmal area of Satpuda Ranges, Maharashtra, India were sampled for ten body sites. Tentative Acinetobacter isolates were confirmed to the genus level by chromosomal DNA transformation assay and to species level using Bouvet and Grimont system. Novel physiological characteristics like pH, temperature and salt tolerance were studied. All strains were screened for production of various enzymes. Results: One hundred and eighteen strains were isolated, which belonged to nine Acinetobacter genospecies. A. haemolyticus was most abundant followed by A. calcoaceticus and A. genospecies 1-3. Higher percentage of Acinetobacter was recovered from skin of nose, Pawara tribe and female volunteers. They showed wide variation in temperature, salt and pH tolerance. Most of the strains could produce enzymes viz, lipase, esterase, urease and amylase. Conclusions: Acinetobacter spp. belonging to nine genospecies were obtained in the present study. Physiological characteristics including high salt, temperature and acidic pH tolerance were helpful to differentiate between the commensal and pathogenic species of Acinetobacter genus.

Key words: Acinetobacter spp., healthy human skin, tribal population, modified chromosomal DNA transformation

Acinetobacter is ubiquitous and has wide distribution in nature. They are natural reservoirs of and account for up to 35-45% of bacteria isolated from human skin. Numerous studies have been reported on composition of bacterial flora of human skin. Some are more specific and resolve Acinetobacter spp. as a major class. Few reports have been published regarding the distribution of various Acinetobacter spp. on human skin in different climatic regions across the world including India, United Kingdom, Germany and Hongkong. Although reports of distribution of Acinetobacter on healthy human skin are available, the physiology of Acinetobacter and its correlation with that of skin microenvironment is not studied. Currently, at least 32 different DNA groups have been reported, but only 17 have been delineated properly. Moreover, there is a continuous addition of new species to genus Acinetobacter from clinical and environmental sources. However, healthy human skin of tribal population not exposed to modern antibiotics is still not explored for the isolation and distribution of Acinetobacter spp. In view of this background, the present investigation was initiated.

Modern-day medicine has not been accepted by most of the tribes who still believe in magico-religious health care systems or in use of folklore medicinal plants found in adjoining areas. This population can be considered to belong to pre-antibiotic era, which has never been exposed to modern antibiotics and thus is an ideal model system to study the distribution of commensal Acinetobacter on truly healthy human skin. Hence, the present study was undertaken with objectives of isolation, biotyping, distribution and physiological characterization of Acinetobacter spp. from the healthy human skin of tribal population of Toranmal area of Satpuda Ranges, Maharashtra, India.

Materials and Methods

Geographical location of tribal area

The Toranmal area chosen for microbiological sampling is situated in Satpuda Ranges, Maharashtra, India (Fig. 1). This area is a home of three tribes: Pawara, Naik and Bhilla. Sampling was done in August 2000 and October 2000. Maximum temperature ranged from 40-45°C in October and minimum was 15-20°C in August. Relative humidity varied from 60 to 80% during sampling.

Selection of tribal population

A total of 53 healthy human volunteers comprising male (n = 29) and female (n = 24) from three tribes, viz Pawara (n = 18), Naik (n = 29) and Bhilla (n = 1), were sampled. Their ages ranged from 20-50 years. Before actual sampling, a general and clinical detail of each volunteer was recorded in a standard
questionnaire form. This form had details of the volunteer such as name, age, sex, tribe, village, pada, skin type (oily/moist/normal), sweating (normal/less/excess), any infection in last 1 year, type of medication taken in last 12 months, alcoholism, hygienic conditions, vaccination, time and date of sample taken and processing of the swab.

Microbiological sampling of skin from tribal population

Microbial samples of skin were collected from 10 different body sites, namely forehead, nose (outer surface), ear (back side of external pinnae), neck, antecubital fossa, axilla, chest, stomach (external naval region), knee joint and back. Skin area was allowed to pre-moisten with 0.85% sterile saline for 2-3 min. A sterile cotton swab pre-moistened with 0.85% saline was rubbed at least for 2-3 min on skin. The swab was directly spread on CLED (Cysteine lactose electrolyte deficient) agar and Holton’s agar media (without cefsulodin and vancomycin antibiotics). All samples were collected and streaked on the spot in the vicinity of burning stove to maintain aseptic conditions. The plates were sealed and kept in a portable incubator-like device at room temperature and brought to the laboratory within 24 h.

Identification and confirmation of genus Acinetobacter using the modified chromosomal DNA transformation assay of Acinetobacter genospecies

Identification of the genus Acinetobacter was done using five preliminary tests, viz. Gram character, motility, oxidase, catalase and capsule staining and were classified as tentative Acinetobacter spp. For confirmation of genus Acinetobacter, chromosomal DNA was purified using Chen and Kuo method as well as Juni’s method and further used for the chromosomal DNA transformation assay. Since we did not obtain adequate lysis of mucoid strains, several modifications were introduced in protocols given by Juni and Chen and Kuo.

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method was suspension of pellet of 1.5 mL bacterial culture in 100 µL of SSC without SDS prior to the addition of 0.5 mL SSC with SDS (0.05%). This supplementary step was adapted in further modifications such as gradual increases in SDS concentration, pH and heating conditions as given in Table 1. The sixth modification introduced was then used for all remaining mucoid strains. For strains not lysed by modified Juni’s method, chromosomal DNA was extracted using modified Chen and Kuo method.9 In original protocol, modification was introduced at lysis step. Pellet was suspended in 100 µL of lysis buffer without SDS by vigorous pipetting. Quantities of lysis buffer with SDS and 5 M NaCl were increased to 300-400 µL and 132 µL depending upon the mucoid nature of the culture. The viscous mixture was centrifuged for 15-30 min/4°C depending upon the time required for packing the lysed mucoid cells during centrifugation. Further steps henceforth were done in per the original protocol.9 Air-dried pellet was suspended in 50 µL of T10 E1 buffer.10 The concentration of DNA was determined by comparison with high DNA mass ladder using agarose gel electrophoresis (Bangalore Genie Pvt Ltd, Bangalore).10

The auxotrophic mutant of A. calcoaceticus BD413 trpE27 was grown on brain heart infusion (BHI; Hi Media Laboratories Pvt. Ltd.) agar for three successive days. About 100 ng of DNA preparation was mixed with loopful of auxotrophic mutant on BHI agar. After 16-18 h of incubation at 28°C, growth was transferred to Acinetobacter minimal medium (AMM) with A. calcoaceticus BD413 trpE27 as a negative control and plate was incubated at 28°C at least for 1 week. In some cases, DNA concentration was increased up to 200-500 ng. Standard cultures used for all experiments included A. calcoaceticus MTCC 1425, A. calcoaceticus MTCC 127, A. calcoaceticus MTCC 1271, A. calcoaceticus BD413 trpE27.

**Biotyping of Acinetobacter genus up to species level using Bouvet and Grimont system**11

Biotyping and characterization of 118 strains of Acinetobacter spp. was done according to Bouvet and Grimont with minor modifications.11 All tests were performed excluding γ-glutamyltransferase, β-xylosidase and utilization of carbon sources such as azelate, histamine and 2,3-butane diol.11 All tests were performed at 37°C and growth was observed every 24 h up to 7 days unless indicated otherwise. Tryptocasien Soy medium was replaced by Luria Bertani medium. Inoculations for all tests were done with 16-18-h-old suspension, OD of which was adjusted to 0.5 McFarland units. Growth at various temperatures was checked in tubes containing 2 mL of Luria broth. Gelatine hydrolysis and haemolysis on Luria agar with 5% whole human blood was recorded after 48 h. Simmon’s citrate agar slants with bromothymol blue (BTB) were used for citrate utilization and results were recorded up to 72 h. Acid from glucose was tested as per the method B of Bouvet and Grimont.11 Utilization of carbon sources was tested in 2 mL of M70 broth supplemented with different carbon sources. Other biochemical tests including growth on eosin methylene

### Table 1: Modifications used for preparation of chromosomal DNA by Juni’s and Chen and Kuo’s method

<table>
<thead>
<tr>
<th>Modification</th>
<th>SDS concentration (%)</th>
<th>pH</th>
<th>Temperature conditions (°C/h)</th>
<th>Efficiency of lysis (%)</th>
<th>Efficiency of transformation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modifications of Juni’s method</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original</td>
<td>0.05</td>
<td>7.2-7.4</td>
<td>60/1</td>
<td>13.3</td>
<td>-</td>
</tr>
<tr>
<td>First</td>
<td>0.05</td>
<td>7.2-7.4</td>
<td>60/1</td>
<td>73.3</td>
<td>77.2</td>
</tr>
<tr>
<td>Second</td>
<td>0.05</td>
<td>8.3</td>
<td>65/1.5</td>
<td>70.0</td>
<td>90.4</td>
</tr>
<tr>
<td>Third</td>
<td>0.05</td>
<td>8.3</td>
<td>70/2</td>
<td>86.6</td>
<td>73.0</td>
</tr>
<tr>
<td>Fourth</td>
<td>0.07</td>
<td>8.3</td>
<td>65/2</td>
<td>00§</td>
<td>-</td>
</tr>
<tr>
<td>Fifth</td>
<td>0.07</td>
<td>8.3</td>
<td>70/2</td>
<td>00§</td>
<td>-</td>
</tr>
<tr>
<td>Sixth</td>
<td>0.08</td>
<td>8.3</td>
<td>70/2</td>
<td>54.2®</td>
<td>26.2</td>
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<tr>
<td>Modified Chen and Kuo method</td>
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<td>Modified Chen and Kuo method®</td>
<td>100®</td>
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<td>41.3</td>
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<tr>
<td>Modified Chen and Kuo method®®</td>
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<td>100®</td>
</tr>
</tbody>
</table>

1Efficiency of lysis was calculated in terms of percentage of strains lysed out of total number of strains taken for chromosomal DNA extraction during that modification; *Efficiency of transformation was expressed as percentage of number of transformants obtained out of number of strains lysed in that modification; §Non-lysed strains in original and three modifications of Juni’s method as they are highly mucoid; ©Non-lysed strains in fourth and fifth modifications + remaining suspected Acinetobacter directly subjected to sixth modification; Strains not lysed in sixth modification tried by modified Chen and Kuo method; Ed strains confirmed as genuine Acinetobacter spp. using DNA preparations isolated by both methods reconfirmed twice by modified Chen and Kuo’s method; *pellet suspended in 100 µL of SSC without SDS by vortexing prior to addition of SSC with SDS. After gentle mixing it was heated in 60°C water bath for 1 h; Warm SSC with SDS at 50-60°C in water bath; equal number of mucoid strains (n = 30) were randomly selected for extraction using Juni’s method in original method as well as modifications.

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blue agar (EMB), nitrate reduction test and oxidation and fermentation were also performed as described elsewhere.12

Novel physiological characteristics of Acinetobacter genospecies

Novel physiological characteristics, such as temperature tolerance (28-53°C), pH tolerance (pH range 5-8) and salt tolerance (range 2-12%), were studied. All tests were done at 37°C except described specifically. They were tested in 2 mL of Luria broth and inoculated with 2 µL of 16-18-h-old suspension, OD of which was adjusted to 0.5 McFarland units. For pH tolerance, Luria broth was prepared in different pH buffers from 4 to 12.13 Tubes were incubated at 37°C up to 8 days and results were recorded every 24 h. Simultaneously, for temperature tolerance, Luria agar plates were spot inoculated and incubated at 28, 37, 41, 44, 50 and 53°C. Growth was observed every 24 h up to 7 days. The mucous nature of Acinetobacter spp. was also studied on Luria agar.

Screening for production of enzymes

All the strains were screened for the production of enzymes including lipase, esterase, lecithinase, DNase, protease, phosphatase, α-amylase and β-galactosidase.12

Results

Features of tribal population from Toranmal

The volunteers’ ages ranged from 20-50 years. From a detailed questionnaire, it was discovered that the hygienic conditions of tribes were poor. All tribal volunteers included in this study had not taken any antibiotic in the past 10 years or never in life and were healthy at the time of sampling.

Characterization of Acinetobacter genospecies and confirmation of genus Acinetobacter by chromosomal DNA transformation assay

Out of the total bacterial population, Acinetobacter comprised the highest (44.8%) number followed by Gram-positive bacteria (32.3%) and other Gram-negative (22.8%) bacteria. Bacterial cultures showing such characteristics as Gram-negative coccobacilli, non-motile, oxidase negative, catalase positive and capsulated or non-capsulated were classified as tentative Acinetobacter spp.1 A total of 261 tentative Acinetobacter strains were isolated and 118 strains were confirmed to be the genus Acinetobacter.

Equal number of mucoid strains (n = 30) was randomly selected for original and first three modifications. Highly mucoid strains that did not lyse were subjected to fourth and fifth modifications. The rest of the highly mucoid strains were directly subjected to sixth modification. The efficiency of lysis was expressed as a percentage of lysed strains out of the total number of strains selected for each modification. As evident from Table 1, the efficiency of lysis by original protocol (13.3%) was remarkably less as compared to average efficiency obtained by the modifications introduced in Juni’s method (47.3%). Nearly 66.6% suspected Acinetobacter strains lysed efficiently. The remaining strains were lysed with 100% efficiency using modified Chen and Kuo’s method. The concentration of chromosomal DNA isolated by modified Chen and Kuo’s method was in the range of 0.5-1 µg/µL.

The reconfirmation of genus Acinetobacter was done twice by modified Chen and Kuo’s method. The efficiency of transformation was expressed as a percentage of strains transformed out of the total number of lysed strains in each modification. The efficiency of transformation using DNA preparations of modified Chen and Kuo’s method (41.8%) was slightly lesser than that of modified Juni’s method (44.4%). However, there was significant difference in the days of incubation required for the transformants to grow on AMM with chromosomal DNA isolated by the two methods. The growth of transformants was observed within 1-2 weeks with crude chromosomal DNA preparation isolated by Juni’s method. However, it was observed within 48-72 h with highly purified chromosomal DNA isolated by Chen and Kuo’s method (Fig. 2).

Biotyping of genus Acinetobacter using Bouvet and Grimont system

One hundred and eighteen strains were characterized and biotyped using Bouvet and Grimont system.11 The basic criterion used in the original system was growth at various temperatures. However, most of the Acinetobacter strains from tribal population could grow well up to 44°C. Hence, this factor was deleted while characterizing Acinetobacter spp. As evident from Table 2, the main criterion was dependent on four tests, including haemolysis, gelatin hydrolysis, acid from glucose and citrate utilization except for A. lwofii.11 Additional tests used for the characterization of each species are specified in Table 2.11 All the groups of Acinetobacter showed 60-100% similarity with the original Bouvet and Grimont system.11

All Acinetobacter strains showed typical dark blue colouration on EMB medium within 24 h; 44% strains...
Table 2: Biochemical characterization of *Acinetobacter* genospecies using Bouvet and Grimont system

<table>
<thead>
<tr>
<th>Characteristics</th>
<th><em>A. haemolyticus</em> (n = 48)</th>
<th><em>A. calcoaceticus</em> (n = 18)</th>
<th><em>A. Iwofii</em> (n = 11)</th>
<th><em>A. genospecies</em> 1-3 (n = 13)</th>
<th><em>A. genospecies</em> 6 (n = 3)</th>
<th><em>A. genospecies</em> 12 (n = 2)</th>
<th><em>A. junii</em> (n = 6)</th>
<th><em>A. johnsonii</em> (n = 1)</th>
<th>Ungrouped (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth at</td>
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<tr>
<td>44°C</td>
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<td>28°C</td>
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</table>

*No. of strains with positive result, †No. of strains with weakly positive result. Remaining strains were negative for given characteristic, bold numbers with cell borders for each *Acinetobacter* spp. represent the key characteristics used for differentiation and numbers with particular signs represents additional characteristics used for differentiation of *A. haemolyticus*; †for *A. calcoaceticus*; ‡for *A. Iwofii*; ‡‡for *A. genospecies* 1-3; *‡for *A. junii* as per the Bouvet and Grimont system.*
Table 3: Species distribution of *Acinetobacter* genospecies on healthy human skin of tribal population of Toranmal area of Satpuda Ranges

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<tr>
<th><em>Acinetobacter</em> spp.</th>
<th>ACF*</th>
<th>Nosea</th>
<th>Neck</th>
<th>Forehead</th>
<th>Axilla</th>
<th>Knee joint</th>
<th>Earb</th>
<th>Stomachc</th>
<th>Back</th>
<th>Chest</th>
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<td>5%/6%/B</td>
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<td>-/3</td>
<td>-/2</td>
<td>1/2</td>
<td>-/5</td>
<td>3/3</td>
<td>-/3</td>
<td>-/4</td>
<td>12%/36%/B</td>
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<td>3/3/-</td>
<td>-/2</td>
<td>-/2</td>
<td>-/-</td>
<td>1/2</td>
<td>-/1</td>
<td>-/2</td>
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<td>5/13/-</td>
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% Recovery$\$$ = 18.64 23.72 12.71 12.71 4.23 3.38 7.62 7.62 3.38 5.93 33.0/66.1/0.8

(Strains) = 12.71/5.93g 12.71/11.01 6.77/5.93 5.93/6.77 0/4.23 0.84/2.54 3.38/4.23 3.38/4.23 0/3.38 2.54/3.38

$^a$No. of strains in Naik tribe, $^b$No. of strains in Pawara tribe, $^c$No. of strains in Bhilla tribe, $^d$Anticubital fossa, $^e$outer surface, $^f$outer surface of the back of the ear, $^g$outer surface around the naval region, $^h$No. of *Acinetobacter* strains in male volunteers, $^i$No. of *Acinetobacter* strains in female volunteers, $^j$percentage of *Acinetobacter* strains in male volunteers, $^k$percentage of *Acinetobacter* strains in female volunteers; $^l$percent recovery represents percentage of *Acinetobacter* strains recovered from particular body site out of total number of *Acinetobacter* strains; $^m$- *Acinetobacter* spp. not isolated.
Table 4: Physiological characteristics of *Acinetobacter* genospecies isolated from human skin of tribal population

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<th>Physiological characteristics</th>
<th><em>A. haemolyticus</em> (n = 48)</th>
<th><em>A. calcoaceticus</em> (n = 18)</th>
<th>Ungrouped (n = 16)</th>
<th><em>A. lwofii</em> (n = 13)</th>
<th><em>A. johnsonii</em> (n = 6)</th>
<th><em>A. genospecies 1-3</em> (n = 11)</th>
<th><em>A. genospecies 6</em> (n = 3)</th>
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</table>

Numbers given in table represent the number of strains with the positive result. Remaining strains are negative for particular characteristic.
reduced nitrate to nitrite, 26.3% strains reduced nitrate to nitrogen gas, whereas 30.3% strains were nitrate negative. Most of strains were fermentative.

Species distribution of Acinetobacter genospecies isolated from healthy human skin of tribal population

Twenty-nine volunteers, 16 (37.2%) males and 13 (30.2%) females, had Acinetobacter spp. on their skin, giving an isolation rate of 67.4%. The isolation rate was higher in October (64.3%) as compared to that of August (35.7%). A result of species distribution of Acinetobacter spp. from healthy human skin of tribal population is shown in Table 3. Female volunteers showed higher recovery (51.6%) as compared to the male volunteers (48.3%). Pawara tribe showed higher recovery (66.1%) followed by Naik tribe (33%).

One hundred and eighty-four strains belonging to nine genospecies of Acinetobacter were identified. A. haemolyticus (40.6%) was most abundant followed by A. calcoaceticus (15.2%). About 13.5% strains were not identified to the species level and were placed under ungrouped category. Higher percentage of Acinetobacter was recovered from nose (23.7%), followed by antecubital fossa (18.6%), neck and forehead (12.7%). Back (3.3%) showed the least per cent recovery. Interestingly, four volunteers, two and one, respectively, showed the presence of four, five and six genospecies.

Novel physiological characteristics of Acinetobacter genospecies

Low salt concentration (2-4% w/v) was suitable for the optimum growth of healthy human skin Acinetobacter of tribal population. However, about 31 strains could grow up to 12% (w/v) salt concentration. A. haemolyticus was the major group showing high salt tolerance in the range of 8-12% (w/v) salt concentration. Results are shown in Table 4. The pH range tolerable by human skin Acinetobacter was 5-8 with an optimum pH 6.5-7.5. All the strains could tolerate pH up to 8. A majority of strains could grow at acidic pH 5.5-6; A. johnsonnii could grow only at pH 6.5-8. The results are shown in Table 4.

Temperature tolerance of healthy human skin Acinetobacter spp. isolated from tribal population up to 53°C was the most important novel characteristic. It was found that 37°C is the optimum temperature for the growth of tribal human skin Acinetobacter strains, whereas most of the strains could grow maximally between the temperature range of 28-44°C. Some strains could resist higher temperatures up to 53°C (n = 37). The results are shown in Table 4. Eighty-five strains out of 118 strains were mucoid.

Enzymes from Acinetobacter spp.

Most of the strains were DNase, lecithinase and protease negative, whereas most strains showed the presence of urease (96.9%), esterase (71.1%) and phosphatase activity (78.8%). They also showed the production of industrially important enzymes, the highest percentages being that of amylases (31.5%), followed by β-galactosidases (27.1%) whereas lipases were the least produced (11.8%). Only one strain of A. haemolyticus was DNase and protease positive. The results are shown in Table 4. The most important fact was that healthy human skin Acinetobacter isolated from tribal population showed esterase (tributyrine hydrolysing) and lipase (olive oil hydrolysing) producing abilities. There was distinct species domination, A. haemolyticus (35.5%) being maximum producers followed by A. calcoaceticus (8.4%). A. johnsonnii and A. genospecies 12 could not produce esterase.

Discussion

Indian tribes constitute roughly 8% of the nation’s total population, which was nearly 68 million according to the 1991 Census. Bhilla and Naik together constitute 48.6% of the total tribal population and Pawara constitutes 51.3% in Toranmal area. Clinical information recorded helped us to understand that tribal population selected was never exposed to modern antibiotics during their lifetime. Till date, healthy staff members working in hospital environments or universities or residing in well-established and highly civilized cities have been sampled for bacterial isolation.\textsuperscript{2-5} Studies done in Indian subcontinent are also restricted to the same population.\textsuperscript{2} Thus, our study constitutes the first report of Acinetobacter isolated from truly healthy human of tribal population not exposed to modern antibiotics.

This investigation included the collection of extensive data including the isolation rate (67.4%), body sites covered (10) for sampling and groups of Acinetobacter genospecies (9) isolated. All these parameters were higher in tribal population as compared to all the previous reports.\textsuperscript{2,5} Our earlier study reported higher percent recovery in male volunteers.\textsuperscript{2} However, it was reverse in case of tribal population and in agreement with that of Hong Kong report.\textsuperscript{5} However, the difference in incidence rates between male and female was not very significant. An incidence rate observed for females was slightly greater than that of male volunteers. There is no association established up till now between the isolation rate and the gender. Higher relative humidity present in male is due to sebum secreted by sebaceous glands.\textsuperscript{14} The sebaceous gland requires androgenic stimulation to produce significant amount of sebum. The levels of most powerful androgens and testosterone are many-fold higher in males and hence higher rates of sebum secretion in males as compared to females.\textsuperscript{14} Similarly, isolation rate of Acinetobacter from the human skin of tribal population was higher in October (65.2%) as compared to August (34.8%). In the state of Maharashtra, the temperature is around 40°C especially in the month of October. Higher prevalence of skin carriage in summer by this genus has been related very well in Hong Kong studies.\textsuperscript{3} Higher percentage of Acinetobacter was recovered from nose, antecubital fossa, neck and forehead (12.7%). This can also be correlated to the higher relative humidity percent and moisture content in these regions. The highest moisture content of forehead and neck may be attributed to sebum secreted by sebaceous glands. The largest glands and the
The greatest density of sebaceous glands are found on the face. A. haemolyticus was a predominant genospecies in case of tribal population, which is recovered in very small numbers (4/112) by Berlau et al. A. lwofii, which comprised a major group in the earlier reports from UK, Germany and India, was restricted to a very small group (16.4%) in this study. Thirteen strains belonging to A. genospp. 1-3 were recovered, which was in agreement with our previous report. The most striking outcome of this study was isolation of new 13.5% strains not identified to the species level and were placed under ungrouped category. This might be because of the use of Holton’s medium without vancomycin and cefsulodin antibiotics in the present investigation that allowed the isolation of newer Acinetobacter spp. The use of Holton’s medium with antibiotics in previous studies might have suppressed the growth of Acinetobacter spp. sensitive to these antibiotics. Moreover, the incidences of colonizing in given body sites by two or more different genospecies were highest in this study as compared to other reports.

Chromosomal DNA transformation assay is used to confirm the genus Acinetobacter. Genus level identification of Acinetobacter can be done using a naturally competent strain of Acinetobacter, which is a tryptophan auxotroph (A. calcoaceticus BD413 trpE27). DNA isolated from any Acinetobacter isolate is capable of transforming this auxotroph to prototroph, whereas DNA from any other bacterial species is unable to do so. This simple assay can be used for rapid identification of large numbers of strains to determine whether or not they are Acinetobacter spp. There was improper mixing of mucoid cells in lysis solutions with SDS while extracting chromosomal DNA from such strains of tentative Acinetobacters by both methods. A simple modification in lysis step allowed the proper mixing of mucous bacterial cell pellet. Hence, it improved the lysis of cells and, therefore, the yield of DNA. In modified Juni’s method, there was a general increase in efficiency of lysis as conditions became more stringent in each modification. This may be mainly because of high temperature and pH together with SDS concentration, which may be denaturing polysaccharides of capsule of mucoid strains. Modified Juni’s method is one step, easiest and less time consuming. However, crude DNA preparation isolated by this method affects the transformation assay in terms of time of incubation. Although modified Chen and Kuo’s method is time consuming, it highly increases the quality of chromosomal DNA and greatly shortens the incubation period for transformation assay. We, therefore, recommend these modifications for efficient chromosomal DNA transformation assay.

Temperature was the basic character used for the classification of Acinetobacter genospecies as per the Bouvet and Grimont system. However, Acinetobacter genospecies isolated from healthy tribal population grew well above 41°C. Not a single genospecies showed 100% similarity with that of the Bouvet and Grimont system. Reliability of the phenotypic tests used for the identification of Acinetobacter spp. is previously compared with that of DNA grouping done by DNA hybridization in Sweden. It shows that, in addition to phenotypic characteristics, DNA hybridization should be proposed for better differentiation of DNA genospecies. 1, 2 and 3. Our study also suggests that, Bouvet and Grimont system is not sufficient for the characterization of Acinetobacter genospecies. Various molecular techniques have advantages over phenotyping as they clearly distinguish within Acinetobacter genospecies. However, this may not be useful for laboratories with limited resources. Soddell (1993) showed that none of the published phenotypically based identification systems are reliable for environmental strains. He proposed that all phenotypically based identification systems are established with clinical isolates and they might differ in varying degrees in biochemical and physiological characteristics with that of isolates from other habitats. This fact was confirmed in our studies, as Acinetobacter isolated from healthy human skin of tribal population showed a range of physiological characteristics.

It was proposed that prolonged survival of Acinetobacter spp. on hospital floors is due to the ability of Acinetobacter to grow at a wide range of temperatures and pH. Earlier studies show that Acinetobacter can tolerate acidic pH. The physico-chemical nature of skin adds to the stress in the form of high salt concentration on its surface from the sebaceous secretions and acidic pH. In addition, human skin as well as bacteria present on skin is exposed to external conditions such as temperature and pH. Even skin parameters such as temperature, pH, evaporative water loss and surface lipid values are affected by seasonal variations. Taking into account these factors, the tolerance of Acinetobacter spp. isolated from healthy human skin of tribal population to a wide range of pH, temperature and salt concentration showed a unique pattern. Nearly 91.5% bacterial strains could tolerate temperature up to 44°C, which may be attributed to maximum temperature recorded during the sampling in October (40-45°C). Most of the strains showed varying mucous nature. The role of mucoid nature in the protection of Acinetobacter in desiccation has been discussed very well. Another possible explanation may be the presence of a polysaccharide capsule formed of l-rhamnose, d-glucose, d-glucuronic acid and mannose, which probably renders the surface of the Acinetobacter strains more hydrophilic which hence tends to survive more in moist/humid conditions. Very few strains in this investigation could produce nitrite from nitrate, which is in agreement with the previous report.

Most of the strains could produce lipase and esterase enzymes. Lipases and esterase produced by Acinetobacter spp. isolated from healthy human skin could help in survival on normal human skin. The stratum corneum of human skin contains a complex mixture of polar lipids produced by epidermal cells and has a surface film of fluid non-polar lipids secreted by sebaceous glands. At the time of final lysis, sebaceous cells release clear oil containing mainly triglycerides. During passage to the human skin, triglycerides in human sebum are partially hydrolysed by bacterial
enzymes to produce free fatty acids. Thus, the production of lipases and esterase by most of Acinetobacter spp. isolated from healthy human skin of tribal population can be correlated to this phenomenon.

Overall, this study provides additional information on the distribution of Acinetobacter spp. on healthy human skin. As the quantity and quality of chromosomal DNA isolated by modified Chen and Kuo’s method was most satisfactory, we highly recommend modified Chen and Kuo’s method for chromosomal DNA isolation from highly mucoid suspected Acinetobacter spp. instead of using crude DNA as in Juni’s method. Novel physiological characteristics showed by Acinetobacter of healthy human skin of tribal population might provide additional information to differentiate between the commensal and pathogenic species of Acinetobacter genus. It also indicates that there might be newer species of Acinetobacter for which molecular systematic studies are needed. Our earlier report on antibiotic susceptibility showed that most of these strains are sensitive to modern antibiotics tested. However, with present-day reports, it is seen that Acinetobacter spp. are known to be multiple drug resistant. Hence, this population might be an ideal model system to study the evolution of antibiotic resistance in bacteria. Further progress in the same area is underway.

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References


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