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Isolation and Characterization of Airborne Bacteria from The Hospital Environment

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Abstract

Microbial flora can be a source of numerous allergic and infective illnesses. Atmosphere is a hostile environment for micro-organisms, this information provides indoor air quality and on associated potential risks in hospitals. Samples were collected from 20 wards by settling plate technique during January-march 2012 in Government Hospital, Pathapatnam, Andhra Pradesh, INDIA. The number of colonies per plate was variable (15-255 colonies). Maximum number of colonies were observed, the organisms isolated during this study were micrococci, aerobic spore bearing bacilli (asb) coagulase negative staphylococci, gram negative bacilli. Micrococci were isolated from 15 wards, ASB from 12 wards, coagulase negative staphylococci from 12 wards, gram Negative bacilli in 2 wards. Antibiotic sensitivity testing was done by Kirby Bauer's method for coagulase negative staphylococci. They were found sensitive to Gentamicin, Kefbactam, ciprofloxacin, ofloxacin, and cloxacillin in descending order of sensitivity.

Keywords

Antibiotic sensitivity Test, Indoor air quality, Kirby Bauer's method, coagulase negative staphylococci, aerobic spore bearing bacilli, Settling plate technique

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INTRODUCTION:

Micro-organisms have evolved special adaptation that favor their survival and dispersal in the atmosphere. Several viral, bacterial and fungal diseases are air borne out breaks. Often follow the changes of environment and ecological factors. Although many microorganisms that grow in the hydrosphere or lithosphere can become airborne. Hospitals are characterized by high infective risk, firstly cause of the compromised immunologic conditions of the patients that make them vulnerable to bacterial, viral, parasitological and fungal opportunistic infections [1]. The bacterial content of air depends of the location that is whether it is outdoor air or indoor air. The bacterial content outdoor air depends on many factors such as the density of human and animal populations, the nature of the soil, the amount of vegetation, the atmospheric conditions, rainfall and sunlight. Humidity, temperature and wind conditions, rainfall and sunlight. Most may contaminate the air seldom survive in the adverse conditions of the outdoor air to result in disease. Bacteria in the upper air consist largely of aerobic spore bearing bacilli and to a much less extent a chromo bacter Sacrcina and Micrococcus [2].

Depending on their sizes, they travel and remain suspended in air or fall to the ground and in the process they evaporate, get converted to very minute particles called droplet nuclei and their fate

depends on the air current in the atmosphere. The bacterial count of indoor air depends of the number of persons, and their bodily movements and the ventilation of that room. Experiments show that the proportion of dust particles and droplet nuclei reaching the lung depend on their sizes. All particles over 5µm are retained in the nose, most of 1µm reach the lung and are retained in the alveoli but below 1µm the proportion retained in the lung diminishes. The ultimate source of common pathogenic organisms is dust derived from human beings [2]. Under favorable conditions, the microorganisms may remain alive for many weeks. Bed clothes are an abundant source of bacteria laden dust. Some microorganisms become airborne as growing vegetative cells, but more commonly, microorganisms in the atmosphere are in the form of spores. Spores are metabolically less active than vegetative cells and are generally better adapted to survival in the adverse conditions of the atmosphere. Spores and vegetative microbial cells face the problems of survival as they become airborne [3].

Most microorganisms can survive a short passage through the atmosphere (Eg: *siella Pneumoniae, Neisseria meningitides, salmonella typhi, vibrio cholerae*). But relatively few survive long distance transport (Eg: *Coryneforms, Gram positive, non-spore forming cacilli, cocci, Actinomycetes, gram negative bacilli, spore forming bacteria, bacillus spps, clostridium spps*) because desiccation can cause microorganisms in the atmosphere to lose viability, particularly in the lower layers of the atmosphere during the day. Many pathogens can cause hospital infections, but those that are able to survive in environment for long periods and develop resistance to antibiotics and disinfectants are particularly important in this respect [2]. The most important pathogens causing hospital infections are *Streptococcus pyogenes, Staphylococcus aureus, Staphylococcus epidermidis* among gram negative bacilli and *Pseudomonas sp*. *E. Coli, Klebsiella, Entero bacter, Proteus* and *Serratia. Salmonella typhimurium, Acentobacter spps*. Tetanus spores can survive in dust for a very long time and may sometimes contaminate cotton, suture material, plaster of paris and other items used in hospitals. Rarely the hospital environment contains *staphylococci*, *Micrococci*, *ASB (Aerobic spore bearing bacilli), Gram negative bacilli.*

MATERIALS AND METHODS:

Air sampling techniques:

The presence of Microorganism in the hospital environment may lead to nosocomial infections. A nosocomial infection is one for which there is no evidence that the infection was present or incubating at the time of hospital admission [4]. In order to prevent such nosocomial infection, sterile conditions have to be maintained in the hospitals. To check the sterility of the hospitals, different techniques have been used one of which is sampling of the air. Air sampling techniques are very much needed in surgical theatres, pharmaceutical industries, food processing industries and in hospitals.

The basic methods employed include:

I. Volumetric impaction sampling
II. Liquid impinger
III. Settling plate technique

I. Volumetric impaction sampling:

Several types of samples are designed to draw a volume of air through a limiting openings the velocity of the air is increased to a point where airborne particles will be impacted onto surfaces. Both solid and liquid surfaces are used [5].

The following are the most commonly used impaction samplers.

1. Slit Samplers:

A number of different commercial types are available.

A. Casella Slit Sampler: The Casella slit sampler employs of metal case fitted with a glass door. The case holds the Petri dish (containing solid nutrient on a turntable rotated by a small electric motor. The sampling rate is one cubic foot per minute when connected to a vacuum source [6, 7].

B. TDL Slit Sampler: The TDL slit sampler houses the Petridish (containing solid nutrient), the turntable and the indicator in a clear plastic housing to the top of which are attached to the slit tube and the indicator. The housing can be completely removed for ready access to the turntable. The sampler operates at the rate of one cubic foot per minute from a remote vacuum source [6, 7].

C. Reyniers Slit Sampler: The Reyniers slit sampler is similar to the TDL and Casella slit samplers. It is available with various timing mechanism and flow meter. The sampling rate is one cubic foot per minute when connected to a vacuum source [5].

d. Mattson/Garvin slit sampler: The Mattson/Garvin air sampler is a portable and completely self-contained slit sampler, including a built in vacuum pump. On operation, air is drawn through a 0.152mm slit at once cubic foot per minute.

2. Cascade of Andersen Sampler:

The Anderesen sampler collects six size graded aerosol particles in a cascaded arrangement of sieves which have smaller diameter holes in each succeeding plate. Agar in Petri dishes are located under each sieve. It samples at the rate of one cubic foot air per minute and will detect one or tens of

thousands of viable particles in a sample. The cascade units are available with or without a vacuum pump. [8, 9, 10]

II. Liquid impinger:

All glass impigners (AGI) have been used for many years. The AGI work on the principle of impacting airborne particles onto liquid surfaces. Impingers, sometimes known as bubblers, are small bottles used with an air sample pump to collect airborne hazards into designated collection liquids for analysis. For personal exposure measurement the impinger mounts in a holster accessory near the breathing zone. For area monitoring the impinger is mounted on the side of a pump with a holder accessory.

- Glass Impingers have graduations in 5ml increments and available with a fritted nozzle to increase contact between the air sample and the liquid.
- PFA impingers are unbreakable, inert to virtually all chemicals and perform well in high temperature and cryogenic applications.

III. Settling plate technic:

This technique is oldest and simplest. The plate is generally an uncovered Petridish containing a blood agar medium. The number of organisms settled depends upon air Sampling which can be carried out with a minimum amount of equipment and training.

Colony counter:

The colony counter has illumination light and magnifying glass to count the colonies. The present study was aimed to detect the aerobic flora present in the hospital environment by using settling plate technique [10, 11]

Enumeration of Microorganisms

Settling plate method:

Blood agar plates were kept at a height of 1 meter and exposed for a period of 30 minutes at the various sampling locations in Government Hospital, Pathapatnam. After exposure to the air, the settling plate is covered and incubated for 24 hrs. at 37^0c. Next day plates were examined for colonies.

Colony counting:

A number of colonies developed on blood agar plates were counted in the colony counter. The organisms were identified by their morphological, staining, and biochemical characteristics.

Microscopic examination:

Simple staining: The smears of bacteria are made on a glass slide. Smears are heated for fixing. Smear was covered with methylene blue for 1-2 minutes. The slide was washed with distilled water to remove excess stain. The slide was dried by blotting and examined using an oil immersion objective. [12]

Gram staining:

The smears of bacteria are made on a glass slide smears are heated for fixing. Smear was covered with crystal violet for 30 seconds. The slide was washed with distilled water. Then 95% ethyl alcohol was added drop by drop until no more colour flows from the smear. Slide was washed with distilled water and blotted dry. Safranin was applied to the smear for 30 seconds. Slide was washed with distilled water and allowed to set dry and examined using an oil immersion objective [13]

Acid fast staining:

The smears of unknown bacterial culture were prepared on slides. Air dried and heat fixed. The smear was flooded with carbol fuchsin and heated the slides to steaming for 3 – 5 minutes from time to time more stain was added to prevent smears becoming air dry. The slides were cooled, washed with distilled water. Decolorized with acid alcohol by adding reagent drop by drop until carbol fushsin failed to wash from the smear. Slide was washed with distilled water. Methylene blue is applied to the smear for 2 minutes and washed with distilled water and allowed to set dry and examine using an oil immersion objective [12].

Biochemical test:

Catalase test:

A drop of NaCl was taken on a clean glass slide. A colony from fresh culture medium was then emulsified. A drop of hydrogen peroxide (H_2O_2) is added to test and not in control and observed for bubbles. Formation of the air bubbles indicates the positive test for *Staphylococci*, if no bubbles occurred it is negative reaction.

Coagulase test:

The test was done as described by 'William and Harper et, (1946)'.

- A clean slide was taken and divided as 2 halves using a waxy pencil.
- On each section, a drop of normal saline is placed with a sterile platinum loop.
- A colony from fresh culture medium was then emulsified.
- A drop of undiluted plasma was added with a loop and mixed gently for 10 seconds.
- If clumps were formed, then the test was considered as positive.
- The second section was kept as control

Tube coagulase Test (Gillespie, 1943):

- One in 10 dilution of the plasma is used. Place 0.5 ml of diluted plasma in test tube and inoculate 0.1 ml of an 18-24 hours broth culture.
- A control was prepared without adding diluted plasma.
- The tubes were incubated at 37^0 c and examined for every hour up to 24 hours for formation of

coagulase. Coagulation indicated the presence of staphylococci. Result may be one of the following types.

1. Plasma has been converted into a stiff gel and remains still even when the tube is inverted.
2. A large clot.
3. Small organized clot.
4. Small unorganized clots, surrounded by clear liquids.

Negative: the plasma remains as a whole and free flowing.

Antibiotic sensitive test:

In this method isolated colonies from an agar medium were touched with a wire loop and the colonies were transferred to a test tube containing 4 ml of sterile peptone water. The tubes were incubated for 2 to 5 hours at 37°C temperature until becomes turbid. Then the nutrient agar plates were placed in an incubator at 37°C for 30 minutes. So as to dry the droplets of moisture on the agar surface. Then the test culture poured on the Petridish and all over the plate. Excess culture was discarded and the plates were dried for 30 minutes. After the inoculums is dried, antibiotic discs were applied with sterile forceps and pressed gently to ensure even contact with the medium plates were incubated in inverted position at 37°C temperature overnight. The next day, diameter of the zone of inhibition around disc was measured with calipers. [11] [14] [15]

The strength of the discs used was as follows:

Antimicrobial agent	Code	Disc Strength
Ciprofloxacin	Cf	5µg
Gentamicin	G	10µg
Erythromycin	E	15µg
Ampicillin	A	10µg
Ofloxacin	Of	10µg
Clozacillin	Cx	5µg
KEFBACTAM	Cm	75µg + 30µg (Cefaperazone + sulbactam)
Methicillin	M	5µg
Tetracycline	T	30µg

RESULT AND DISCUSSION:

Assessment of viable microbial count using air Sampler:

The bacteria present in various locations in Government Hospital, Pathapatnam were estimated by settling plate method using blood agar medium. [10, 16]

The results were as follows:

	Source	No. of colonies	No. of organisms Identified
1	Emergency Operation Theatre	52	*1)micrococci* *2)ASB*
2	S_1 ward	189	*1)micrococci* *2) Gram-ve Bacilli*
3	S_2 ward	130	*1)micrococci* *2)staphylococci*
4	S_3 ward	208	*1)micrococci* *2) ASB*
5	S_4 ward	150	*1)micrococci* *2) staphylococci*
6	A – operation theatre	49	*1)staphylococci* *2) ASB*
7	B – operation theatre	41	*1)staphylococci* *2) ASB*
8	Septic Ward	124	*1)micrococci* *2) Gram-ve stout Bacilli* *3) ASB*
9	Paediatric surgical theatre	245	*1)micrococci* *2) staphylococci*

			3) ASB
10	Orthopaedic Ward	212	*1)micrococci* *2) ASB*
11	Burns ward	39	*1)micrococci* *2) ASB*
12	Skin and STD ward	81	*1)micrococci* *2) staphylococci* *3) ASB*
13	Gynaec theatre	253	*1)micrococci* *2) staphylococci* *3) Cocci& Bacilli*
14	Cardiology ward	231	*1)micrococci* *2) staphylococci*
15	Casualty 1	167	*1)ASB* *2) staphylococci*
16	Casualty 2	118	*1)micrococci* *2) ASB*
17	Open air	110	*1)micrococci* *2) staphylococci*
18	Microbiology head of the department room	19	*1)micrococci* *2) ASB*
19	Culture room in microbiology	87	*1)micrococci* *2) staphylococci*
20	Media room	103	*1)micrococci* *2) staphylococci* *3) ASB*

(ASB- aerobic spore bearing bacilli)

IDENTIFICATION OF THE TEST ORGANISM:

1) Cultural, morphological and biochemical characteristics of bacterial sample:

Test for Identification	Characters
1) Cultural characters	Blood agar
Shape	Circular
Colour	White
Elevation	Convex
Opacity	Opaque
Consistency	Soft
2) Morphological characters	
Acid Fast Staining	Non Acid Fast
Gram Staining	Gram +Ve
Cell Shape & Size	Cocci, <1µ In Size
Cell Arrangement	Grape Like Clusters
3) Biochemical tests:	
Catalase	positive
Coagulase	Negative

Based on the cultural characters, morphological characters and biochemical tests the test organism was identified as coagulase negative *staphylococci* [18].

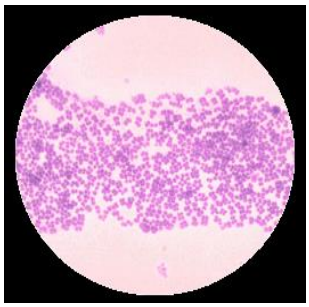
Staphylococci

2. Cultural, morphological and biochemical characteristics of bacterial sample

Test for Identification	Characters
1) Cultural characters	Blood agar
Shape	Circular
Colour	white
Elevation	Convex
Consistency	Soft
2)Morphological Characters	
Acid Fast staining	Non Acid Fast
Gram Staining	Gram+Ve
Cell shape & Size	Cocci,<1 µ in size
Cell arrangement	Grape like Clusters
3) Biochemical tests:	
Catalase	Positive

Based on the cultural characters, morphological characters and biochemical tests the test organism was identified as *micrococcus* [17].

Micrococcus

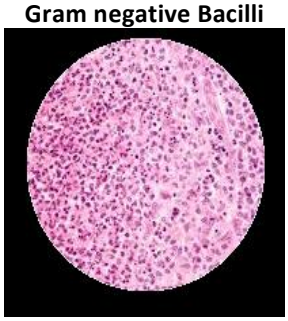


3) Cultural, morphological and biochemical characteristics of bacterial sample:

Test for Identification	Characters
1) Cultural characters	Blood agar
Shape	Round
Colour	Pale white
Elevation	Raised
Opacity	Translucent
Consistency	Mucoid
2)Morphological Characters	
Acid Fast staining	Non Acid Fast
Gram Staining	Gram -Ve
Cell shape & Size	Cocci, 2-3 µ in size
Cell arrangement	Nonspecific arrangement

Based on the cultural characters, morphological characters and biochemical tests the test organism was identified as *Gram-Negative Bacilli.* [15, 19, 20]

Gram negative Bacilli

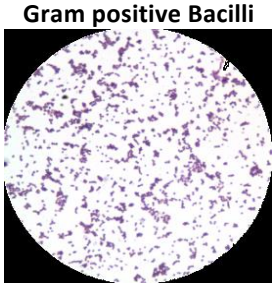


4) Cultural, morphological and biochemical characteristics of bacterial sample:

Test for Identification	Characters
1) Cultural characters	Blood agar
Shape	Round
Colour	Pale white
Elevation	Raised
Opacity	Opaque
Consistency	Soft
2)Morphological Characters	
Acid Fast staining	Non Acid Fast
Gram Staining	Gram +Ve
Cell shape & Size	Cocci, 3-4 µ in size
Cell arrangement	Nonspecific arrangement

Based on the cultural characters, morphological characters the test organism was identified as *Gram-Positive Bacilli* [20, 21]

Gram positive Bacilli



5) Cultural, morphological characteristics of bacterial sample

Test for Identification	Characters
1) Cultural characters	Blood agar
Shape	Irregularly round shape
Colour	Grayish white
Elevation	Flat
Consistency	Rough
2)Morphological Characters	
Acid Fast staining	Non Acid Fast
Simple staining	Rods in Chains
Gram Staining	Gram +Ve
Cell shape and Size	Cocci, 5-10 µ in size
Cell arrangement	Arranged in chains

Based on the cultural characters, morphological characters the test organism was identified as *Bacilli.*

The antibiotic sensitivity pattern of coagulase negative staphylococci in percentage:

Organism	Cf	G	E	A	Of	Cx	CM
Coagulase negative staphlycocci	79%	100%	31%	47%	74%	53%	100%

Abbreviations:
Cf - Ciprofloxacin
G - Gentamicin
E - Erythromycin
A - Ampecillin
Of - ofloxacin
Cz - cloxacillin
Cm - kefbactam

19 strains of coagulase negative *staphylococci* were isolated from various wards as mentioned above and sensitivity pattern was done by Kirby – Bauer's method. All strains were sensitive to Gentamicin and Kefbactam and showed 79% sensitivity to ciprofloxacin, 74% sensitivity to Ofloxacin, 53% sensitivity to Cloxacillin. Most strains showed resistance to Ampicillin (47%) and Erythromycin (31%).

RESULT AND DISCUSSION:
The Hospital Environment is generally fully laden with microorganisms because large number of patients undergo treatment for various diseases large number of patients undergo treatment for various disease for long periods. Persons get infected in hospital direct with the source or by means of airborne transmissions. Organism enter the environment in the form of droplet nuclei and droplets from the nose, mouth or skin of the patients, attendants and hospital staff and may cause nosocomial infection in persons whose immunity is lowered. Catheterization, respiratory intubation, I.V. fluids lines may carry organisms from the external surface of the patient to internal organs. Pathogens may also be present in antiseptic lotions and ointments.
Organisms like *staphylococci, micrococci, Pseudomonas, E. coli, Klebsella*, *Tetanus spores* are mostly present in the hospital environment these pathogens are capable of infection in healthy persons and may even cause death. To prevent nosocomial infections, it is necessary to test the hospital environment frequently. The present study is an attempt to study the "Aerobic Flora in hospital during the period Jan & March 2012. Samples were collected from various wards by the method of setting plate technique. Particles present in air settled on the surface of the blood agar plates after exposure for 30 minutes. The plates were incubated for 24 hours at 37°C. Next day plates were examined for colonies; colonies were counted in colony counter. The organisms were identified by their cultural, morphological, staining and biochemical characters.

CONCLUSION:
In this study the most commonly isolated organisms were coagulase negative *Staphylococci, micrococci, Gram negative bacilli, Gram positive bacilli* and *aerobic spore* bearing bacilli. Antibiotic sensitivity of the coagulase negative *staphylococci* was done by Kirby-Bauer's method. All strains of coagulase negative staphylococci were sensitive to Gentamicin and Kefbactam, and showed 79% sensitivity to ciprofloxacin, 74% sensitivity to ofloxacin, 53% sensitivity to cloxacillin. Most strains showed resistance to ampicillin (47%) and Erythromycin (31%).

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REFERENCES:
[1] Lucia Bonadonna,Rossella Briancesco,Anna Maria C occia, 'Analysis of Microorganisms in Hospital Environments and Potential Risks', Department of Environment and HealthIstituto Superiore di SanitàRomeItaly.
[2] R. Ananthanarayan & C.K Jayaram paniker, text book of microbiology, orient Longman (publishers) 5th edition, 1996.
[3] Atlas and bharath, microbial ecology fundamental & application, an imprint of Addison weley Longman, in (publishers) 4th edition, 2000.
[4] T. GRACE EMORI* AND ROBERT P. GAYNES, CLINICAL MICROBIOLOGY REVIEWS, Oct. 1993, p. 428-442
[5] Joseph r. Luciano, air contamination control in hospitals, plenum press, new yard and London (publishers) 1977.
[6] Lawrence B. Hall. M.S. and Herb) ert M. Decher. M.S.S.E. PROCEDURES APPLICABLE TO SAMPLING OF THE ENVIRONMENT FOR HOSPITAL USE VOL. 50. NO. 4, A.J.P.H 1960.
[7] J Ochei, A Kolhatkar, Medical laboratory science theory and Practice, 2000.
[8] Geo f. Brooks, Janet s. Butel & Stephen a. Morse; jawetz, melinick & Adel berg's medical microbiology, mc graw hill (publishers) 22nd edition, 2002

[9] JUNG (M.Y.), KIM (J.S.) and CHANG (Y.H.): Bacillus acidiproducens sp. nov., vineyard soil isolates that produce lactic acid. Int. J. Syst. Evol. Microbiol., 2009, 59, 2226-2231

[10] Cappuccino, J.G Sherman N. (2007) microbiology manual, 7th edition, TATA Art printers, India.

[11] James G. Cappuccino, Natalie Sherman. "Microbiology A Laboratory Manual" seventh edition. 1-216. James G. Cappuccino, Natalie

[12] Dr. Geeta Sumbali, Dr R S Mehrotra, Principles of Microbiology, 2009.

[13] Subhash K. Mohan Gram Stain: Looking beyond Bacteria to Find Fungi in Gram Stained Smear, A laboratory guide for medical microbiology, 2009.

[14] Sherman. "Microbiology a Laboratory Manual" 3rd edition. The Benjamin kummings publishing company, inc. c. Cervantes, Bacteria1l introduction with chromate, ton Leeuwenhoek, 59,229.

[15] JUNG (M.Y.), KIM (J.S.) and CHANG (Y.H.): Bacillus acidiproducens sp. nov., vineyard soil isolates that produce lactic acid. Int. J. Syst. Evol. Microbiol.,2009, 59,2226-2231.

[16] John D.T and James H.J (1999) General casideration. Manual of clinical microbiology 7th edition. Murray P.R Baron E.J pfaller M.A, Ternover F.C, Yalken R, American society for microbiology Washington DC, P. 1469-1473.

[17] Sims GK, Sommers LE, Konopka A (1986). "Degradation of Pyridine by Micrococcus luteus Isolated from Soil". Appl Environ Microbiol 51 (5): 963–968

[18] Harris L.G, Foster S.J, Richards S. G. (2002). "An introduction to Staphylococcus aureus, and techniques for identifying and quantifying S. aureus adhesins in relation to adhesion to biomaterials: review". European cells and materials 4: 39–60

[19] SHIVAJI (S.), CHATURVEDI (P.), SURESH (K.), REDDY (G.S.N.), DUTT (C.B.S.), WAINWRIGHT (M.), NARLIKAR (J.V.) and BHARGAVA (P.M.): Bacillus aerius sp.nov., acillus aerophilus sp. nov., Bacillus stratosphericus sp.nov.and Bacillus altitudinis sp. Nov., isolated from cryogenic tubes used for collecting air samples from high altitudes. Int. J. Syst. Evol. Microbiol., 2006, 56, 1465 -1473.

[20] NAKAMURA(L.K.): DNA relatedness of Bacillus brevis Migula 1900 strains and proposal of Bacillus agri sp. nov., nom. rev., and Bacillus centrosporus sp. nov., nom. rev. Int. J. Syst.Bacteriol., 1993, 43, 20-25.

[21] ALBERT (R.A.), ARCHAMBAULT (J.), ROSSELLÓ-MORA (R.), TINDALL (B.J.) and MATHENY (M.): Bacillus acidicola sp. Nov., a novel mesophilic, acidophilic species isolated from acidic Sphagnum peatbogs in Wisconsin. Int. J. Syst. Evol. Microbiol.,2005,55, 2125-2130.