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ORIGINAL ARTICLE

Isolation and Diagnosis of *Staphylococcus lentus* from Different Operation Theater Hospitals

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ABSTRACT

Objectives: Hospital environment play critical role in nosocomial infection, because this environment contains various microorganisms. A frequency of increased pathogenic bacteria in the environment of the hospital is associated with a surroundings rise in different types of nosocomial infections.

Methods: A total of 500 samples were collected from environmental samples and clinical samples were distribution on Basra hospitals during the period from October 2016 to February 2017. All isolates were subjected to the cultural, microscopical ,biochemical examination and identification by 16S ribosomal DNA (16S rDNA) and Sequencing of 16S rDNA.

Results: It was found that 204 (40.8%) of the total bacterial agents in all surgical theaters with a significant statistical superiority in the prevalence of positive bacteria 187 (91.66%)in different surgical theaters. The study included the diagnosis of 53 isolates (91.37%) Gram positive cocci formulations giving 47 isolates (81.03%) of Staphylococcus species as follows: 17 isolation of *Staphylococcus lentus* (29.31%). *Staphylococcus lentus* was Sensitive to Benzyl penicillin, Oxacilin, Gentamicin, Teicoplanin, Tigecycline, Trimethoprin and Nitrofurantion,Levofloxacin, Rifampicin. While *S.lentus* was Resistant to Erythromycin, Levofloxacin**,** Vancomycin, but *S. lentus* was Intermediate to Moxifloxacin Clindamycin, Teteacycline , Fosfomycin.

Conclusion: The emergence of a new type of bacteria *Staphylococcus lentus* which has not been isolated from hospitals previously. *Staphylococcus lentus* are considered as a pathogenic bacteria and it has the ability to pathology as well as multiple resistance to antibiotics.

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INTRODUCTION

Hospital environment play critical role in nosocomial infection, because this environment contains various microorganisms. A frequency of increased pathogenic bacteria in the environment of the hospital is associated

with a background rise in different types of nosocomial infections[1].

The center for disease control and prevention (CDC) appoints healthcare associated infections as infections that patients acquire during the cure for other conditions or workers of the health care acquire while carry out their activities within health care framings[2]. Healthcare associated infections is happened because of the environmental organism depends on many factors. The protection of healthcare associated infections depends on several factors including effective cleaning, sterilization and disinfection proceedings[2].

Bacteria are the most common pathogens responsible for nosocomial infections. Some belong to natural flora of the patient that cause infection only when the immune system of the patient becomes prone to infections. The common bacteria related with infections of nosocomy are *Staphylococci, Escherichia coli*, and *Pseudomonas*. Although, many pathogens can cause hospital infection but those that are capable to pull out in the hospital environment for long periods and also resist to disinfections are particularly substantial in this respect. Gram positive bacteria were classifid as a main reason of nosocomial infection, with *Staphylococcus aureus*; comonly known as staph; in the center of these causes Staphylococci are opportunistic pathogens although they are normal flora of cutaneous organisms existent in the environment[3]. Staphylococcus is found in about 25-30% in the skin and the nose of healthy people (i.e. asymptomatically), and occasionally they cause infections. These bacteria are the most common cause of skin infection in the USA. *Staphylococcus aureus* has developed resistance over the past 20 years to many antimicrobial agents which are used generally in the USA[4]. Another causitive agent of nosocomail infection is *S. epidermidis* and group D of Streptococci are also reported as nosocomaial infection agent[5]. *Staphylococcus lentus* is a Gram-positive, oxidase-positive, coagulase-negative, member of genus *Staphylococcus* consisting of clustered cocci. The species was originally distributing as a subspecies; its name is an integration derived from *Staphylococcus sciuri* subsp. *Lentus*[6]. *Staphylococcus lentus* is capable of free-living existence, since then it can grow on inorganic nitrogen salts as alone source of nitrogen, and it has been isolated from soil, water, sand, and marsh grass[7,8]. *S. sciuri* can be found as a colonizing organism in humans, with low transporter rate in the nasopharynx, skin and urogenital tract. However, members of this set may also be important pathogens of human in charge of endocarditic peritonitis, septic shock, infections of urinary tract, pelvic inflammatory disease, most frequently, and wound infections[6].

MATERIALS AND METHODS

Collection and Transport

Five hundred swabs air and surface samples were possessed from all operating theatres of a three Hospitals of AL-Basrah city, Abn AL Baetar Hospital, AL Basrah General Hospital. AL-Sadr Hospital. in different. The contamination of bacteria in a surgical theater is evaluated by using settle plate and swab method.

(i) Settle Plate Method: Performed taking air samples with a settle plate method. We used Petri dishes containing blood agar and MacConkey agar, where they are then transferred to the surgical operating room in plastic bags and the number of the sample, the site during the stage and the time of the sample collection. An openning Petri dishes were placed in several selected locations of the operating rooms, and were exposed for 1-2 h to air before and after surgical operations (pre and post sterilization). After that the petri dishes were covered and transported to the lab in plastic bags where they were incubated at 37C◦ for 24-48 hours[9].

(ii) Swab Method: In this method sterilized swabs were used to collect samples from hand and nosal of the surgeons and works also from floors , equipment, beds mask and machinery hall, that are used in the work and wounds of patients before and after surgical operations (pre and post sterilization), described these samples are dully and transported to the laboratory than culture and streaked on Blood agar , MacConkey agar, and Mannitol salt agar plates. The plates of culture along with those exposed in air were incubated at 37 °C under aerobic conditions for 24-48 hrs. After incubation the colonies were enumerated and identification of isolates was performed[9].

Diagnosis of Isolates Bacterial

Morphology of the bacterial Colony: Colonies that grow on the selective media were further identified through the study of their morphological characteristics beginning by Gram stain and appearance under light microscope.

Bacteriological identification and Antibiotic susceptibility testing of bacterial isolates: Microorganism were identified based on culture characteristics, Gram staining properties and biochemical test (confirmed with the automated microbiological system vitek2)[10] .

Genetic identification

Genomic DNA extraction: This procedure was done by using commercially genomic DNA mini Kit (Geneaid, Korea). The procedure was explained in details in user´s manual.

16S ribosomal DNA (16S rDNA): Specimens of the bacteria were identified by using PCR to amplify universal bacterial 16S rDNA primers 27F 5'-AGAGTTTGATCCTGGC-3' and 1492 R 5'-GGTTACCTTGTTACGACTT-3'[11]. And to give a final volume of 50 µl were used for PCR amplification, F Primer 2.5 µl, R Primer 2.5 µl, DNA template 3 µl, Nuclease-free water 17 µl were added in Master Mix tube 25 µl and the reactions were heated to Initial denaturation 92˚C for 2 min, following by 30 cycle of denaturation 94˚C for 30 Sec, Annealing 51.8˚C for 45 Sec, Extension 72˚C for 1.5 min . A Final extension was carried out at 72˚C for 5 min. Five µl of 1 kb DNA ladder, 5 µl of 16S rDNA produced from PCR were electrophoreses for 1.5h in (50V) in casting tray with 2% agarose gel prepared in 1×TBE, containing 0.5 µl of

ethidium bromide in 100 ml of agarose solution. The products were viewed under UV light system, the band of 1500bp was indicative to 16S rDNA.

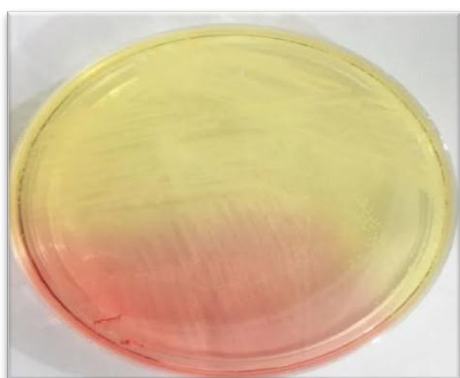
Sequencing of 16S rDNA: The 16S rDNA gene sequence was treated then analyzed using Basic Local Alignment Search Tool 'BLAST' to investigation for homologous sequences in the National Center for Biotechnology Information database (NCBI) http://www.blast.ncbi.nlm.nih.gov. The bacterial sequence was identified by proper it with a sequence with the highest identity score from the GenBank database[12].

RESULTS

Positive microbial isolates

Two hundred and four (40.8%) of the total bacterial agents isolated and The study included the diagnosis of 53 isolates (91.37%) Gram positive cocci formulations giving 47 isolates (81.03%) of Staphylococcus species as follows : 17 isolation of *Staphylococcus lentus* (29.31%). *Staphylococcus lentus* showed significant differences ($p < 0.01$) than among other groups of bacteria. *Staphylococcus lentus* appeared to be fermented for lactose and are not analyzed when they grow high on blood , as show in Figure 1 (a and b). The diagnosis of these bacteria was performed by PCR from the new strains of these bacteria emerged when sent to the gene bank and recorded on it as in the Table 1 note that this diagnosis was supported by Biochemical test, as show in Table 2.

A



B

Figure 1: **A- *Staphylococcus lentus* on Blood agar**
B- On Manitol Salt agar

Identification of bacteria by 16S rRNA Gene

Agarose gel electrophoresis patterns show DNA bands of bacterial isolates as showed in Figure 2.

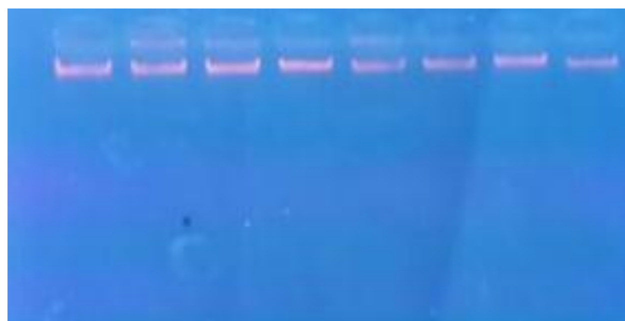


Figure 2: **Agarose gel electrophoresis patterns show DNA bands of bacterial isolate**.

Amplification of 16S rRNA Gene

16SrRNA gene was successfully amplified, according to modified PCR condition, and the size of amplicon was that they were of different lengths compared with 2k bp marker and molecular weight of 1500 bp band Figure 2. Only 45µl from each amplicons were sent to Sango Biotech (Shanghai) Co, Ltd, as showed in Figure 3.

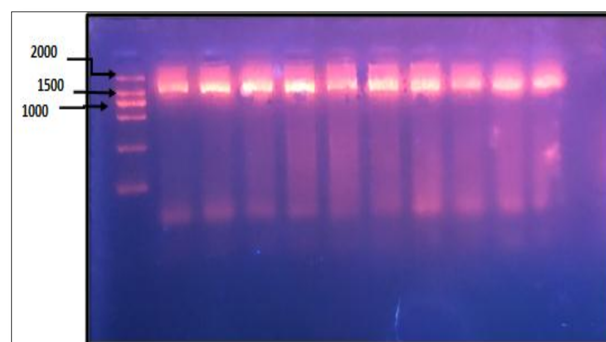


Figure 3: **Agarose gel electrophoresis patterns show amplified PCR products of 16Sr RNA corresponding to a 1500 bp band when compared to the molecular ladder. Lane 1molecular DNA marker (100-2000) bp.**

Five isolates were selected from the groups of bacteria that were identified by biochemical tests, In this study isolations were identified based on the phenotypic properties confirmed by the 16S rDNA sequence, where a single discrete PCR amplicon band of 1500bp of 16S rDNA was observed when determined on agarose gel as shown in Table 1 , 2 and Figure 4.

Identification by biochemical tests by used (confirmed with the automated microbiological system vitek2)

Identification of *Staphylococcus lentus* depending on morphological and biochemical reactions, the results shown in Table 3.

Table 1: **16S rDNA gene sequences, number of isolates from bacteremia and % identical to Genbank strains.**

Nucleotide sequence	length	% Identical to Genbank strain
GCTATAATGCAGTCGAGCGAACAGATGAGAAGCTTGCTTCTCT GATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTACC TATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGA TAATATATTGAACCGCATGGTTCAATGTTGAAAGACGGTTTCG GCTGTCACTTATAGATGGACCCGCGCCGTATTAGCTAGTTGGT AAGGTAACGGCTTACCAAGGCAACGATACGTAGCCGACCTGAG AGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCC TACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGC CTGACGGAGCAACGCCGCGTGAGTGATGAAGGTCTTAGGATCG TAAAACTCTGTTGTTAGGGAAGAACAAATTTGTTAGTAACTGA ACAAGTCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTAC GTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCG GAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTCTTAAGTCT GATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAAC TGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGT AGCGGTGAAATGCGCAGAGATATGGAGGAACACCAGTGGCGAA GGCGGCTCTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCGT GGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAA ACGATGAGTGCTAAGTGTTAG	**1425**	**99%**

Table 2:**The result of sequenced amplicon of *Staphylococcus lentus*.**

No	Bacterial species	length	% Identical to Genbank strain	**Accession number of isolate
1	*Staphylococcus lentus*	1425	99%	TAM2 MH880119
2	*Staphylococcus lentus*	1425	99%	TAM3 MH880120
3	*Staphylococcus lentus*	1428	99%	TAM6 MH880123
4	*Staphylococcus lentus*	1459	99%	TAM8 MH880125
5	*Staphylococcus lentus*	1459	99%	TAM9 MH880126

** The accession number new under processing may be change identification (ID).

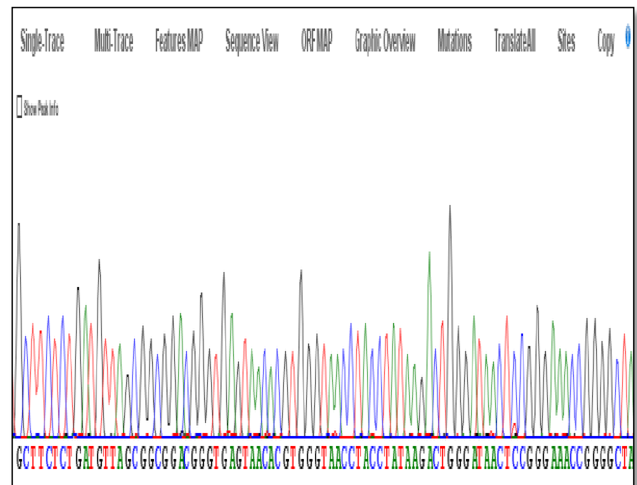


Figure 4: **The beak quality of sequenced amplicon of *Staphylococcus lentus***

Table 3: **The result of biochemical reactions of *Staphylococcus lentus* by used automated microbiological system vitek2.**

Type of bacteria	AMY	PIPLC	DXYL	ADH1	BGAL	AGLU	APPA	CDEX	ASPA	BGAR	AMAN	PHOS	LEUA	PROA	BGURR	AGAL	PYRA	BGUR	ALAA	TYRA	DSOR	URE	POLYB	DGAL	DRIB
Staphylococcus lentus	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	+
	PUL	DRAF	O129R	SAL	DTRE	ADH2S	OPTO	ILATK	LAC	NAG	DMAL	BACL	NC6.5	NOVO	NC6.5	DMAN	DMNE	MBDG	PUL	DRAF	O129R	SAL	DTRE	ADH2S	OPTO
	-	-	-	+	+	-	+	-	+	-	-	+	-	+	+	+	-	-	-	-	-	+	+	-	+

Table 4: **The result of Antimicrobial Sensitivity of *Staphylococcus lentus* by used automated microbiological system vitek2.**

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Cefoxitin	NEG	-	Teicoplanin	1	S
Benzyl penicillin	<= 0.03	S	Vancomycin	>= 32	R
Oxacilin	2	S	Teteacycline	8	I
Gentamicin	<= 0.5	S	Tigecycline	<= 0.12	S
Tobramycin	<= 1	S	Fosfomycin	16	I
Levofloxacin	>= 8	R	Nitrofurantion	<= 16	S
Moxifloxacin	4	I	Fusidic acid	2	S
Erythromycin	>= 8	R	Rifampicin	1	S
Clindamycin	1	I	Trimethoprin / sulfamethoxazole	<= 10	S
Linezolid	2	S			

S: Sensitive **R** : Resistant **I** : Intermediate

Antibiotic susceptibility testing by used (confirmed with the automated microbiological system vitek2)

Staphylococcus lentus isolates that were resistant or sensitive to commonly used (confirmed with the automated microbiological system vitek2). The antibiotic sensitivity and resistance patterns of the isolates was demonstrated on Table 4 . *Staphylococcus lentus* was Sensitive to Benzyl penicillin, Oxacilin, Gentamicin, Teicoplanin, Tigecycline, Trimethoprin and Nitrofurantion, Levofloxacin, Rifampicin. While *S.lentus* was Resistant to Erythromycin, Levofloxacin**,** Vancomycin, but *S. lentus* was Intermediate to Moxifloxacin Clindamycin, Teteacycline and Fosfomycin .

Discussion

Results of total microbial isolates showed statistically significant differences between the prevalence of positive and negative gram stain bacteria in all surgical theaters with a significant statistical superiority in the prevalence of positive bacteria in different surgical theaters.The reason may be that they can withstand drought conditions[13,14]. *Staphylococcus lentus* showed significant differences ($p < 0.01$) than among other groups of bacteria, these results do not match what he has reached[13]. Although *Staphylococcus lentus* are fermented for lactose and this characteristic is similar to bacteria, they are small colonies. They also grow on the blood agar, but they are Non-blood analyzer, while *Staphylococcus* auras are a blood analyzer. Although the diagnosis by Amplification of 16S rRNA Gene the most

accurate in all its details, but we need the biochemical characteristics for the purpose of identifying this bacteria more, the presence of these bacteria is dangerous because *Staphylococcus lentus* are considered as a pathogenic bacteria[15].

16S rRNA gene was successfully amplified. Relatively, modified PCR condition similar results were obtained by[16,17] because of the existence of 16S rDNA in all bacteria and its stable function over a lot of evolutionary time is large enough to obtain information purpose[18].

Staphylococcus lentus are considered as a pathogenic bacteria and it has the ability to pathology as well as multiple resistance to antibiotics. As well, many bacteria, including Staphylococcus, are becoming resistant to multiple antibiotics[15]. The high prevalence of resistance to levofloxacin, erythromycin, vancomycin and clindamycin was not unexpected as compared to proceding studies on CoNS in poultry litter in the United States[19,20].

Conclusions

The emergence of a new type of bacteria *Staphylococcus lentus* which has not been isolated from hospitals previously, *Staphylococcus lentus* are considered as a pathogenic bacteria and it has the ability to pathology as well as multiple resistance to antibiotics.

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REFERENCES

1. Muhammad U.K., Isa M.A. and Aliyu Z.M. "Distribution of potential nosocomial pathogens isolated from environments of four selected hospital in Sokoto, North Western Nigeria". J. Microbiol. Biotech. Res. 2013; 3 (1):139-143.
2. Kabbin J.S., Shwetha J.V., Sathyanarayan M.S. and Nagarathnamma T. "Disinfection and sterilization techniques of operation theater: a review". International Journal of Current Research. 2014; 6(5): 6622-6626.
3. Iyer A.P., Baghallab I., Albaik M. and Kumosani T. "Nosocomial Infections in Saudi Arabia Caused by Methicillin Resistance Staphylococcus aureus (MRSA)". Clin. Microbial. 2014; 3(3):146. doi: 10.4172/2327-5073.1000146 .
4. Pantosti A., Sanchini A. and Monaco M. "Mechanisms of antibiotic resistance in *Staphylococcus aureus".* Future Microbiol. 2007; 2(3): 323-334. DOI:10.2217/17460913.2.3.323.
5. Yagoub S.O. and Agbash A. El. "Isolation of Potential Pathogenic Bacteria from the Air of Hospital-Delivery and Nursing Rooms". Journal of Applied Sciences. 2010; 10(11): 1011-1014. DOI: 10.3923/jas.2010.1011.1014
6. Stepanović S., Dakić I., Martel A., Vaneechoutte M., Morrison D., Shittu A., Ježek P., Decostere, A., Devriese, L. and Haesebrouck F. "A comparative evaluation of phenotypic and molecular methods in the identification of members of the Staphylococcus sciuri group". Systematic and Applied Microbiology. 2005; 28 (4): 353–357. DOI:10.1016/j.syapm.2005.02.001.
7. Kloos W.E., Schleifer K.H. and Smith R.F. "Characterization of Staphylococcus sciuri sp. nov. and its subspecies". Int. J. Syst. Bacteriol. 1976; 26(1): 22–37.
8. Kloos W.E. "Natural populations of the genus Staphylococcus". Annu. Rev. Microbiol. 1980; 34(1): 59–592.
9. Singh K., Dar F.A and Kishor K. "Bacterial contamination in operating theaters of district hospital Budgam in Kashmir division". International Journal of Pharma Sciences and Research. 2013; 3(2): 62-63.
10. Skucaite N., Peciuliene V., Vitkauskiene A. and Machiulskiene V. "Susceptibility of endodontic pathogens to antibiotics in patients with symptomatic apical periodontitis". J. Endod. 2010; 36(10):1611-1616. DOI:10.1016/j.joen.2010.04.009.
11. Miyoshi K., Tsukumo H., Nagami T., Siomi H. and Siomi M.C. "Slicer function of Drosophila Argonautes and its involvement in RISC formation". Genes & Dev. 2005; 19(23): 2837–2848. DOI:10.1101/gad.1370605.
12. Jenkins C., Ling C.L., Ciesielczuk H.L., Lockwood J., Hopkins S., Mchugh T.D., Gillespie S.H., Kibbler C.C. and Jenkins C. "Detection and Identification of Bacteria in Clinical Samples by 16S rRNA Gene Sequencing : Comparison of Two Different Approaches in Clinical Practice". Journal of Medical Microbiology. 2012; 61(4): 483–488. DOI:10.1099/jmm.0.030387-0.
13. Fu Shaw L., Chen L.H., Chen C.S.H., Wu H.H., Lai L.S.H., Yin Chen Y.Y. and Fu D.W. "Factors influencing microbial colonies in the air of operating rooms". BMC Infectious Diseases. 2018; 18(1) :4. DOI:10.1186/s12879-017-2928-1.
14. Rathore L., Khatri P.K., Meena S., Bora A., Sharma N. and khullar Sh."Investigation of Bacterial Counts in Air at Different Wards of a Tertiary Care Hospital". IJSRM. 2015; 3(7): 3396-3400.
15. Pennington H. "Millennium bugs". Biologist (London). 2000; 47(2), 93-95.
16. Kaur A., Kaur M., Samyal M.L. and Ahmed Z. "Isolation, characterization and identification of bacterial strain producing amylase". J. Microbiol. Biotech. Res. 2012; 2(4) :573-579.
17. Sultan N.S. and Sinha M.P. "16S rDNA based identification of bacteria in the organophosphates treated agricultural soil". Int. J. Appl. Sci. Eng. Res. 2012; 1(2): 212-223.
18. Janda J.M. and Abbott Sh.L. "16S rRNA Gene Sequencing for Bacterial Identification in the Diagnostic Laboratory: Pluses, Perils, and Pitfalls". J. of Clinical mico. 2007; 45(9): 2761–2764. DOI:10.1128/JCM.01228-07.
19. Simjee S.U., Jawed H., Quadri J. and Saeed S.A. "Quantitative gait analysis as a method to assess mechanical hyperalgesia modulated by disease-modifying antirheumatoid drugs in the adjuvant-induced arthritic rat". Arthritis Res Ther. 2007; 9(5):R91.
20. Bhargava K. and Zhang Y. "Multidrug-resistant coagulase-negative Staphylococci in food animals". Journal of Applied Microbiology. 2012; 113(5): 1027-1036. DOI:10.1111/j.1365-2672.2012.05410.x.