Scientific Research Publishing

Journal of Biosciences and Medicines, 2019, 7, 138-148
http://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Identification of Microbes in Patients with Lumbar Disc Herniation

N. D. Withanage[1*], S. Pathirage[2], S. Perera[3], H. Peiris[4], L. V. Athiththan[4]

[1]Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka
[2]Medical Research Institute, Colombo 8, Sri Lanka
[3]The Central Hospital, Colombo 8, Sri Lanka
[4]Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka
Email: *withanagend@sjp.ac.lk

How to cite this paper: Withanage, N.D., Pathirage, S., Perera, S., Peiris, H. and Athiththan, L.V. (2019) Identification of Microbes in Patients with Lumbar Disc Herniation. *Journal of Biosciences and Medicines*, **7**, 138-148.
https://doi.org/10.4236/jbm.2019.76009

Received: May 13, 2019
Accepted: June 22, 2019
Published: June 25, 2019

Abstract

Apart from the conventional factors, recent evidences have suggested that lumbar disc herniation (LDH) is also associated with microbes, which is completely ignored in the management of patients with disc prolapse and disc degeneration. Therefore, the present study was carried out to identify the different microorganisms in subjects with LDH. Subjects (n = 101) who were confirmed for LDH with Magnetic Resonance Imaging and undergoing lumbar discectomy, were recruited in this study. Standard protocols for disinfection of the skin and surgical instruments were adhered. Skin scrapings, muscle biopsies and portion of the intervertebral disc were transferred into individually labeled Robertson's cooked meat enrichment broth for anaerobic identification. Remaining portions of the excised disc material and muscle biopsy were taken for aerobic identification. Anaerobic isolates were identified using Gram stain and catalase test while the species identification was done by RapID ANA II ID kit. Gram stain, catalase test, DNase test and coagulase tests were used for identification of aerobic bacteria. Study confirmed 6/101 disc cultures (6%) with positive anaerobes and 12 disc cultures with coagulase negative *Staphylococci* spp. Among the anaerobes, two disc cultures were identified as *Propionibacterium acnes* and one as *Gemella morbillorum.* Due to slow growth, other three anaerobic cultures were not confirmed. However, they resembled the colony morphology of Gram positive bacilli. None of the control samples (skin and muscles) had any positive growth. The present study adds to the literature confirming the role of microorganisms in LDH. Present study newly identified *Gemella morbillorum* in the intervertebral tissue in addition to the previously reported microorganisms associated with LDH.

Keywords

Lumbar Disc Herniation, *Propionibacteruim acnes*, Coagulase Negative *Staphylococci* spp, *Gemella morbillorum*

1. Introduction

Although there are numerous determinants for lower back pain (LBP), lumbar disc herniation (LDH) is regarded as the major contributing factor [1] [2]. LDH and lumbar disc degeneration (LDHD) are multifaceted where age, gender, severe mechanical as well as physical loading, trauma, obesity, strenuous sporting activities, vibrations and smoking were attributed as the major contributing factors [1] [3] [4] [5].

Apart from the mentioned conventional factors, recent studies have suggested that genetic polymorphism and microbes also play an important role in LDH. Evidences further suggest that role of microbes has been completely ignored in the management of patients with disc prolapse and disc degeneration. Studies have proven that antibiotic protocol has been effective in management of lumbar disc herniated patients [6]. According to the literature, it was believed that LBP associated with LDH arises not only due to neural compression resulting from prolapsed disc, but also due to bacterial infection that occurs around the herniated disc area [7] [8]. Recent studies have identified the presence of microorganisms in association with LDH. Most of the studies have reported *Propionibacterium acnes* (*P. acnes*) as the major organism isolated associated with LDH and LDHD [6]-[12]. All the above reported studies have isolated *P. acnes* as the most predominant microorganism with the isolation range of 7% - 53%, and some studies have found different strains of *P. acnes* as well [6]. It is stated that poor blood supply and low oxygen tension around the intervertebral disc tissues favor the ideal growth conditions for anaerobes such as *P. acnes* [13]. Cultivation, isolation and identification of anaerobes from clinical specimens is a difficult task as brief exposure to atmosphere during sampling procedure can reduce many anaerobic microorganisms preventing them from proper identification [14]. Although the association of microorganisms with LDH has been regarded as a paradigm shift, still there is not enough data on clinical research regarding this novel finding. Further, there is a lack of proper contamination controls during the culturing of intervertebral disc tissues for detecting microorganisms. Thus, present study was carried out to identify the presence of microorganisms in the disc tissue of patients with LDH and to differentiate them. Exceptionally, the study has used skin scrapings and muscle biopsies as control specimens for the microbial cultivation.

2. Methods and Material

2.1. Study Design and Setting

Microbial analysis was carried out at the Medical Research Institute (MRI), Co-

lombo, Sri Lanka. Patients were recruited from a hospital in the Colombo district, Sri Lanka. Ethical approval was obtained from the Ethics Review Committee of Faculty of Medical Sciences (No-29/14), University of Sri Jayewardenepura, Sri Lanka and MRI Colombo, Sri Lanka (No-52/14). This study was conducted in the period of May 2015 to January 2018. After explaining the study protocol, informed written consent was obtained from all individual participants.

2.2. Study Population

The study involved 101 subjects who were admitted for lumbar discectomy from a selected hospital in Sri Lanka. Subjects were confirmed for LDH by consultant neurosurgeon and consultant radiologist with the aid of Magnetic Resonance Image. Patients with previous history of fever, chronic or acute infections/inflammation during past two weeks and patients who have taken antibiotics treatments during last two weeks of recruitment were excluded from the study.

2.3. Biopsy Collection

In order to prevent bacterial contamination of the skin, stringent aseptic procedures were followed; this included cleaning the skin of the surgical field pre-operatively two times with 70% (v/v) isopropyl alcohol and three times with povidone iodine solution prior to skin incision.

Following skin disinfection, skin scrapings, portion of muscle biopsies and surgically removed disc material were collected and transferred into individual Robertson's cooked meat enrichment broth (RCM) using sterile forceps for anaerobic studies. Other portion of the excised disc material and muscle sample were collected into separate sterile containers for aerobic studies.

2.4. Aerobic Isolation

Disc and muscle samples were inoculated into blood agar, chocolate agar, and MacConkey agar (Oxoid, UK). Blood agar and MacConkey agar plates were incubated at 37˚C for 48 hours while chocolate agar plates were incubated in candle jar (5% - 10% CO_2) at 37˚C for 48 hours. Direct Gram stain was performed for all three plates. In addition, a portion of disc sample and a muscle biopsy were enriched using Brain heart infusion broth (BHI) for 24 hours. After 24 hours of incubation, enriched samples were sub cultured on blood agar, chocolate agar and MacConkey agar and incubated further for 24 hours at 37˚C. Blood agar and MacConkey agar plates were incubated at 37˚C, while chocolate agar plates were kept in candle jar in 5% - 10% CO_2. Muscle sample was used as the control specimen.

2.5. Anaerobic Isolation

Disc samples in RCM enrichment broth were incubated for 48 hours at 37˚C and sub cultured further on blood agar, Bacteriods Bile Esculin agar (BBE) and Brucella

Blood agar (BBA) (Oxoid, UK). They were incubated at 37˚C anaerobically using anaerobic packs (Oxoid, UK). Anaerobic plates were read at day two and seven. Cultures that were positive on anaerobic incubation were subjected to aero tolerance test to confirm the presence of aerobes. The above said anaerobic culture procedures were followed for the control samples (skin scrapings and muscle biopsy) to rule out the possibility of contamination from skin bacteria. Gram stain was performed on the isolates.

2.6. Aerotolerance Test

Growth observed under anaerobic conditions, was sub cultured on blood agar plates and incubated at 37˚C for 24 hours for aerotolerance test.

2.7. Identification

1) Gram staining

Gram staining was performed for direct smears of muscle biopsy and disc biopsy in aerobic isolation procedure and for positive cultures in both anaerobic and aerobic isolations [15].

2) Biochemical tests

Catalase, coagulase and DNase tests were used to identify bacteria inanaerobic and aerobic cultures [15].

2.8. Identification of Aerobes

Staphylococci spp identification was done by Gram staining, catalase, DNase and tube coagulase test.

2.9. Identification of Anaerobes

After identifying the anaerobes with Gram stain and catalase test further identification of isolates was performed using RapID ANA II ID kit (remel, USA) which is a commercially prepared microsystem, designed specifically for the identification of medically important anaerobes. The RapID ANA II ID system offers the advantages of four hours aerobic incubation and identification based on enzymatic degradation of chromogenic substrates by preformed bacterial enzymes.

3. Results

In the present study, 18/101 (18%) patients' herniated disc material were positive for the presence of either aerobic or anaerobic microorganisms, where 6/101 (6%) were positive for anaerobic microorganisms and 12% were positive for the presence of coagulase negative *Staphylococcus* spp (CoNS) (**Table 1**).

In the identified CoNS, four were aerobes and eight were facultative anaerobes. Among the anaerobic cultures only three (n = 3) samples were identified where two were *P. acnes* (2%) (**Figure 1** and **Figure 2**) while the other was identified as *Gemella morbillorum.* Although three other cultures showed positive

Table 1. Types of bacteria identified in herniated disc cultures.

Isolated species of Microorganism	Herniated discs (n 101)
Propionibacterium acnes (anaerobes)	2
Gemella morbirllorum (anaerobes)	1
Not identified (anaerobes)	3
Coagulase negative *Staphylococci* spp	12

Figure 1. Blood agar culture plate of *Propionibacteruim acnes.*

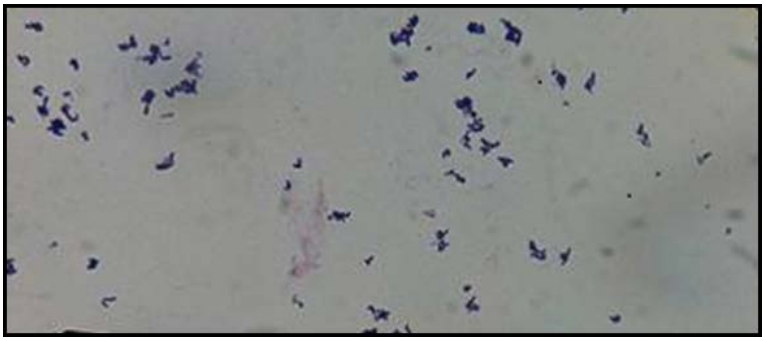

Figure 2. Photomicrograph of *Propionibacteruim acnes* following Gram stain (×40).

growth for anaerobic isolations, they were not identified further, due to slow growth of colonies, but they resembled the morphology of Gram positive bacilli on Gram staining. However, none of the anaerobic positive herniated disc cultures had positive anaerobic isolations in the control specimens (skin scrapings and muscle biopsies) (Table 2). None of the muscle biopsies used as controls had any positive growth for aerobic isolations as well.

4. Discussion

Chronic LBP associated with LDH is considered as the commonest cause for work place absence in many countries. Although it was suggested that numerous

Table 2. Comparison of anaerobic positive herniated disc cultures with the controls.

Type of organism	Patient ID	Isolated microorganisms	Skin scrapings	Muscle biopsies	Herniated discs
Anaerobic	Patient 1	*Propionibacterium acnes*	-	-	+
	Patient 2	*Propionibacterium acnes*	-	-	+
	Patient 3	*Gemella morbirllorum*	-	-	+
	Patient 4	Not identified – Gram + bacilli	-	-	+
	Patient 5	Not identified – Gram + bacilli	-	-	+
	Patient 6	Not identified – Gram + bacilli	-	-	+

(–): organisms absent, (+): organism present.

physical, mechanical, behavioral and environmental factors attributed to chronic LBP associated with LDH, recent studies have revealed that microorganisms and genetic factors play an important role in LDH.

This study finding adds to the literature on microbes in excised intervertebral disc tissue following lumbar discectomy. Rollason *et al.* have found 38% (24/64) patients with *P. acnes* in herniated disc tissue, while they have identified 5/64 (8%) as CoNS. Our study finding also had both species but 12% of the discs were positive for CoNS and only 2% of the disc samples had *P. acnes*. According to Rollason *et al.* there were two patients with both *P. acnes* and CoNS. But none of our study samples had mixed colonies.

Similar to the present, study Rollason *et al.* also had excluded patients who had taken antibiotic treatments for past two weeks. In addition they have excluded patients with previous epidural injections or back surgery. However, present study included 15 patients with repeated back surgery. However, a different study conducted revealed that 53% (10/19) with positive disc cultures had not undergone pre-operative epidural injections, and seven subjects with negative tissue culture (n = 17) had previous epidural injections validating that previous epidural injection is not a major source of bacterial infection at the in tervertebral disc [8]. In the present study patients with previous epidural injections were not excluded as our aim was to identify the presence of microbes in LDH.

To rule out the contamination Rollason *et al.* have used five separate disc material from each patient and CoNS was found only in one disc material (1/5) confirming the presence of CoNS was not due to any contamination [6]. However in this present study muscle and skin biopsy were used as control to rule out the contamination and none of them were positive for microbes.

Stirling *et al.* conducted a study on 36 patients who underwent microdiscectomy and they found that 16 (44%) patients had *P. acnes* in the disc cultures after long term incubation (7 days and 21 days), while there were two patients with positive disc cultures for CoNS and one disc culture was positive for the presence of *Corynebacterium propinquum* [8]. However, in the present study the incubation period was two and seven days. Hence three samples with slow

growth were unable to be identified. Stirling *et al.* concurrently recruited 14 control patients who suffered from other spinal disorders such as, scoliosis (n = 3), trauma (n = 3), myeloma (n = 2) and degenerative disc disease (n = 6) and none of these disc cultures had positive cultures even after long term incubation. Stirling *et al.* also cultured 11 disc tissues that were large enough directly on the Blood agar plates (Oxoid, UK) without enrichment and 6/11 (55%) of these cultures were positive for *P. acnes*.

Albert *et al.* involved 61 patients who underwent primary surgery at a single spinal level for lumbar disc herniation excluding the subjects who had received any antibiotic treatments for two weeks; but this study also did not include subjects with past history of back surgery, however, in our study among the 15 patients who underwent repeated lumbar disccetomy, only three patients (3/15) were positive for the presence of microbes. Albert *et al.* have sub cultured the evacuated disc material onto Columbia blood agar plates (Oxoid, UK) at 37˚C for seven days in aerobic and anaerobic conditions. After seven days the isolated colonies were further sub cultured onto same agar media for 24 hours at 37˚C. *P. acnes* were identified following Analytical Profile Index (API) biochemical analysis using the Rapid ID 32A kit (bioMerieux) and by Polymerase Chain Reaction amplification of 16S rDNA. According Albert *et al.*, disc culture study, microorganisms were positive in28/61 patients, while 26 of them were positive with anaerobic microorganisms. *P. acnes* (24/61) was the predominant anaerobic microorganism and only two patients were identified with CoNS (anaerobe) (2/61). Further, four (4/61) patients had two microorganisms (one aerobic and other anaerobic) isolated in single disc culture [7].

Some other studies have also reported similar findings of microorganisms in herniated disc tissue following lumbar discectomy. Corscia *et al.* have reported that 71% of the study subjects (n = 30) presented with positive microbial cultures; where 36% was CoNS and 18% was *P. acnes* [7]. Agarwal *et al.* found that 10/52 (19%) subjects had positive microbial cultures for the excised disc after single level microdiscectomy where *P. acnes* accounted for 13% [7] [10]. All the above reported studies have isolated *P. acnes* as the most predominant microorganism with the isolation range of 7% - 53%, whereas in this study CoNS was isolated as the major bacteria with 12 % isolation rate. Isolation of *P. acnes* (2%) in our study is low compared to other findings except Corscia *et al.* Further, in this study three Gram positive bacilli (3/101) resembled the colony appearance of *P. acnes*, but could not be confirmed by the RapID ANA II identification kit. The pure sub culture colonies did not show sufficient growth after 48 hours of incubation (Recommended incusbation for the kit was 18 - 24 hour, but less than 72 hours) to prepare the inoculum to achieve a visual turbidity equal to a #3 McFarland turbidity standard. According to kit manufacturer's instructions suspensions with significantly less turbid than #3 McFarland standard will result in aberrant reactions. As such this could have contributed to low percentage of *P. acnes* in the present study. Further, *P. acnes* needed extended length of incu-

bation. However, the current study findings add to others findings [6] [7] [8] with regard to isolation rate of CoNS (12 %) in extruded disc tissue; with only exception to the study of Corscia *et al.*, where the isolated CoNS was 36% [7]. A study enrolled 80 patients with LDH and underwent discectomy identified 21 disc cultures with *P. acnes* and 5 disc cultures with CoNS [12].

Furthermore the present study identified *Gemella morbillorum* in herniated disc tissue following lumbar discectomy [16] which highlighted as an important finding to the existing; literature on microorganisms and LDH. *Gemella morbillorum* is a Gram positive, anaerobic, non-motile, non-spore forming catalase negative coccus which appears as commensal flora in female genital tract, intestinal tract and upper respiratory tract. Tunniclifin 1917 first described it; and was initially named as *Streptococcus morbillorum* and later transferred to the present genus in 1988 according to DNA homology, physiological characteristics and 16S RNA catalogue [17].

Although infections caused by *Gemella morbillorum* organism in central nervous system are limited, there were few reported isolations in brain abscesses. Even though there are several cases reported in relation to endocarditis and septic shock [18] [19] [20] [21] there had not been any reported studies on *Gemella morbillorum* in relation to disc herniation, except for the case report by Withanage *et al.* [13]. However, Garcia-Borders, Luis *et al.* has reported on spontaneous pyogenic spondylodiscitis and epidural abscess in vertebral fracture [22]. According to the literature, *Gemella* has been reported in patients with pneumonia, sinusitis, gynaecological infections, septic arthritis and infections of the eye [20].

Intervertebral disc tissue is a highly avascular tissue with low oxygen tension and low pH. The blood supply and nutrition to intervertebral disc are via diffusion through vertebral end plate [23] [24] [25]. In the present study the anaerobes were *P. acnes* (2/101) and *Gemella morbillorum* (1/101). Although the present study identified 12 CoNS, four of them were aerobically harvested, while the rest (8/12) was only isolated in anaerobic RCM enrichment, suggesting that these microorganisms could be facultative anaerobic CoNS (8/12). Predominance of anaerobes in the disc cultures might be attributed to low oxygen and low pH which provides the ideal growth conditions for anaerobes [7].

Query on intraoperative contamination could be excluded as all possible standard precautions was taken in this study, including pre-operative skin disinfection, disinfection and sterilization of surgical instruments and specimen transferring utensils in addition to strict sterile conditions during surgical invasion. Similar skin disinfection procedures have been used in other studies as well [7] [9] [26]. Studies have also stated that mixed cultures should be present if there are any contaminations. But present study only yielded monocultures confirming the findings cannot be due to contaminations. Stirling *et al.* has conducted a study with similar skin disinfection procedures and showed that skin contamination was zero in the control patients who underwent similar spinal

procedure other than disc herniations [7] [8] [9] [10].

Further in this study skin scrapings and muscle biopsies were collected as controls from the same site under strict sterile conditions. Culture of control samples (muscle biopsy and skin scrapings) done concurrently with disc cultures were negative, out ruling the possibilities of any contamination. However, skin scrapings have not been considered as controls for aerobic bacteria due to practical limitations of cosmetic effect of the surgical scar. In the CoNS isolations none of the muscle biopsies were positive for CoNS. This further confirms that presence of bacteria in the intervertebral disc could not be due to contaminations.

5. Limitations

RapID ANA II ID system uses a numerical approach. This is a probability method for the identification as such; it cannot identify strains outside its existing database. Therefore isolates which fall outside the existing database and unknowns may incorrectly be assigned to the identification taxon.

6. Conclusion

Present study findings add to the reported studies on the role of microorganisms in LDH, confirming that there could be a possible role of microorganisms in LDH leading to symptoms of chronic lower back pain and inflammation. This study newly identified *Gemella morbillorum* in the intervertebral tissue following lumbar discectomy. In addition, this was the first study to use muscle biopsies and skin scrapings concurrently as control specimens.

Acknowledgements

Financial assistance by University Grants Commission, Sri Lanka (Grant no-UGC/DRIC/PG/2013) and Medical Research Institute, Sri Lanka (Grant no-52-2014).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Eskola, P.J., Lemmelä, S., Kjaer, P., Solovieva, S., Männikkö, M., Tommerup, N., Lind-Thomsen, A., Husgafvel-Pursiainen, K., Cheung, K.M. and Chan, D. (2012) Genetic Association Studies in Lumbar Disc Degeneration: A Systematic Review. *PLoS ONE*, **7**, e49995. https://doi.org/10.1371/journal.pone.0049995 http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0049995

[2] Hirose, Y., Chiba, K., Karasugi, T., Nakajima, M., Kawaguchi, Y., Mikami, Y., Furuichi, T., Mio, F., Miyake, A., Miyamoto, T., Ozaki, K., Takahashi, A., Mizuta, H., Kuba, T., Kimura, T., Tanaka, T., Toyama, Y. and Ikegawa, S. (2008) A Functional Polymorphism in THBS2 That Affects Alternative Splicing and MMP Binding Is

Associated with Lumbar-Disc Herniation. *American Journal of Human Genetics*, **82**, 1122-1129. https://doi.org/10.1016/j.ajhg.2008.03.013

[3] Colombini, A., Brayda-Bruno, M., Lombardi, G., Croiset, S.J., Vrech, V., Maione, V., Banfi, G. and Cauci, S. (2014) Fok I Polymorphism in the Vitamin D Receptor Gene (VDR) and Its Association with Lumbar Spine Pathologies in the Italian Population: A Case-Control Study. *PLoS ONE*, **9**, e97027. https://doi.org/10.1371/journal.pone.0097027

[4] Adams, M.A. and Roughley, P.J. (2006) What Is Intervertebral Disc Degeneration, and What Causes It? *Spine*, **31**, 2151-2161. https://doi.org/10.1097/01.brs.0000231761.73859.2c

[5] Eser, B., Cora, T., Eser, O., Kalkan, E., Haktanır, A., Erdogan, M.O. and Solak, M. (2010) Association of the Polymorphisms of Vitamin D Receptor and Aggrecan Genes with Degenerative Disc Disease. *Genetic Testing and Molecular Biomarkers*, **14**, 313-317. https://doi.org/10.1089/gtmb.2009.0202

[6] Rollason, J., Mcdowell, A., Albert, H.B., Barnard, E., Worthington, T., Hilton, A.C., Vernallis, A., Patrick, S., Elliott, T. and Lambert, P. (2013) Genotypic and Antimicrobial Characterisation of *Propionibacterium acnes* Isolates from Surgically Excised Lumbar Disc Herniations. *BioMed Research International*, **2013**, Article ID: 530382. https://doi.org/10.1155/2013/530382

[7] Albert, H.B., Lambert, P., Rollason, J., Sorensen, J.S., Worthington, T., Pedersen, M.B., Nørgaard, H.S., Vernallis, A., Busch, F. and Manniche, C. (2013) Does Nuclear Tissue Infected with Bacteria Following Disc Herniations Lead to Modic Changes in the Adjacent Vertebrae? *European Spine Journal*, **22**, 690-696. https://doi.org/10.1007/s00586-013-2674-z

[8] Stirling, A., Worthington, T., Rafiq, M., Lambert, P.A. and Elliott, T.S.J. (2001) Association between Sciatica and *Propionibacterium acnes*. *The Lancet*, **357**, 2024-2025. https://doi.org/10.1016/S0140-6736(00)05109-6

[9] Arndt, J., Charles, Y.P., Koebel, C., Bogorin, I. and Steib, J.P. (2012) Bacteriology of Degenerated Lumbar Intervertebral Disks. *Clinical Spine Surgery*, **25**, E211-E216. https://doi.org/10.1097/BSD.0b013e318269851a

[10] Agarwal, V., Golish, S.R. and Alamin, T.F. (2011) Bacteriologic Culture of Excised Intervertebral Disc from Immunocompetent Patients Undergoing Single Level Primary Lumbar Microdiscectomy. *Journal of Spinal Disorders and Techniques*, **4**, 97-400. https://doi.org/10.1097/BSD.0b013e3182019f3a

[11] Urquhart, D.M., Zheng, Y., Cheng, A.C., Rosenfeld, J.V.R., Chan, P., Liew, S., Hussain, S.M. and Cicuttini, F.M. (2015) Could Low Grade Bacterial Infection Contribute to Low Back Pain? A Systematic Review. *BMC Medicine*, **13**, 13. https://doi.org/10.1186/s12916-015-0267-x

[12] Tang, G., Wang, Z., Chen, J., Zhang, Z., Qian, H. and Chen, Y. (2018) Latent Infection of Low-Virulence Anaerobic Bacteria in Degenerated Lumbar Intervertebral Discs. *BMC Musculoskeletal Disorders*, **19**, 445. https://doi.org/10.1186/s12891-018-2373-3

[13] Albert, H.B., Sorensen, J.S., Christensen, B.S. and Manniche, C. (2013) Antibiotic Treatment in Patients with Chronic Low Back Pain and Vertebral Bone Edema (Modictype 1 Changes): A Double-Blind Randomized Clinical Controlled Trial of Efficacy. *European Spine Journal*, **22**, 697-707. https://doi.org/10.1007/s00586-013-2675-y

[14] Holland, J.W., Hill, E. and Altemeier, W. (1977) Numbers and Types of Anaerobic Bacteria Isolated from Clinical Specimens since 1960. *Journal of Clinical Microbi-*

ology, **5**, 20-25.

[15] Prescott, L.M., Harley, J.P. and Klein, D.A. (2002) Laboratory Exercises in Microbiology. 5th Edition, McGraw-Hill Corporation, Pennsylvania, 45-47, 170-175.

[16] Withanage, N.D., Athiththan, L.V., Perera, S., Pathirage, S. and Peiris, H. (2017) Isolation of *Gemella morbillorum* in Herniated Intervertebral Disc Tissue in a Lumbar Discectomy Patient: A Case Report. *Journal of Biosciences and Medicine*, **5**, 99-104. http://www.scirp.org/Journal/PaperInformation.aspx?PaperID=74846 https://doi.org/10.4236/jbm.2017.53010

[17] Kilpper-Bälz, R. and Schleifer, K. (1998) Transfer of *Streptococcus morbillorum* to the Genus Gemella as *Gemella morbillorum* comb. nov. *International Journal of Systematic Bacteriology*, **38**, 442-443. https://doi.org/10.1099/00207713-38-4-442

[18] Liberto, M.C., Matera, G., Puccio, R., Barbieri, V., Quirino, A., Capicotto, R., Guadagnino, V., Pardatscher, K. and Focà, A. (2006) An Unusual Case of Brain Abscess by *Gemella morbillorum*. *Japanese Journal of Infectious Diseases*, **59**, 126.

[19] Akiyama, K., Taniyaso, N., Hirota, J., Iba, Y. and Maisawa, K. (2001) Recurrent Aortic Valve Endocarditis Caused by *Gemella morbillorum*. Report of a Case and Review of the Literature. *Japanese Circulation Journal*, **65**, 997-1000. https://doi.org/10.1253/jcj.65.997

[20] Vasishtha, S., Isenberg, H.D. and Sood, S.K. (1996) *Gemella morbillorum* as a Cause of Septic Shock. *Clinical Infectious Diseases*, **22**, 1084-1086. https://doi.org/10.1093/clinids/22.6.1084

[21] Zakir, R.M., Dehneh, A.A., Dabu, L., Kapila, R. and Saric, M. (2004) Mitral Bioprosthetic Valve Endocarditis Caused by an Unusual Microorganism, *Gemella morbillorum*, in an Intravenous Drug User. *Journal of Clinical Microbiology*, **42**, 4893-4896. https://doi.org/10.1128/JCM.42.10.4893-4896.2004

[22] García-Bordes, L., Repiso, J.A.A., Soler, J.C.C., Juan, C., Fabregas, C., Montauti, M., *et al.* (2010) An Unusual Case of Spondylodiscitis. *Spine*, **35**, E167-E171. https://doi.org/10.1097/BRS.0b013e3181be5ea2

[23] Antoniou, J., Steffen, T., Nelson, F., Winterbottom, N., Hollander, A.P., Poole, R.A., Aebi, M. and Alini, M. (1996) The Human Lumbar Intervertebral Disc: Evidence for Changes in the Biosynthesis and Denaturation of the Extracellular Matrix with Growth, Maturation, Ageing, and Degeneration. *Journal of Clinical Investigation*, **98**, 996. https://doi.org/10.1172/JCI118884

[24] Humzah, M. and Soames, R. (1988) Human Intervertebral Disc: Structure and Function. *The Anatomical Record*, **220**, 337-356. https://doi.org/10.1002/ar.1092200402

[25] Smith, L.J., Nerukar, N.L., Choi, K.S., Harfe, B.D. and Elliot, D.M. (2011) Degeneration and Regeneration of the Intervertebral Disc: Lessons from Development. *Disease Model &Mechanisms*, **4**, 31-41. https://doi.org/10.1242/dmm.006403

[26] Fritzell, P., Bergström, T. and Welinder-Olsson, C. (2004) Detection of Bacterial DNA in Painful Degenerated Spinal Discs in Patients without Signs of Clinical Infection. *European Spine Journal*, **13**, 702-706. https://doi.org/10.1007/s00586-004-0719-z