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THE EFFICACY OF ANTI-MICROBIAL AGENTS AGAINST *ACTINOMYCES* SPECIES ISOLATED FROM PATIENTS WITH DENTAL CARIES

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ABSTRACT

Introduction: Destruction of teeth is a serious problem in oral and general health of the human being, and dental caries is one of the major defects of dental hard tissue.

Objectives: The objective of this research was to isolate *Actinomyces* species from dental caries using polymerase chain reaction technology, and to examine the *in vitro* antibiotic resistance of isolates using a specific 16S rRNA primer to investigate the effect of several anti-bacterial agents, such as amikacin, gentamicin, ciprofloxacin, amoxicillin, ceftriaxone, cefixime, vancomycin, and erythromycin against *Actinomyces* species.

Material and methods: Eighty samples from dental carious lesions were collected and tested for the current study between March and June, 2021. GP-ID cards with 64 biochemical tests were utilized in the automated Vitek-2 Compact system to confirm bacterium isolates. The basis of polymerase chain reaction technology was DNA polymerase's capacity to manufacture a new DNA strand. Kirby-Bauer disc diffusion method was applied to examine the *in vitro* antibiotic resistance of isolates taken from dental caries using a specific 16S rRNA.

Results: *Actinomyces meyeri* and *A. viscosus* were detected with 16S rRNA genes using PCR technique. All 18 (100%) *A. meyeri* identified by Vitek-2 system provided positive results for specific 16S rRNA gene at 519 bp, and all 19 (100%) *A. viscosus* identified by Vitek-2 system provided positive results for specific 16S rRNA gene at 787 bp. The use of selective antibiotic tests, such as amikacin, gentamicin, ciprofloxacin, amoxicillin, ceftriaxone, cefixime, vancomycin, and erythromycin demonstrated the impact of antibiotics against *A. meyeri* and *A. viscosus* isolates.

Conclusions: The detection of *Actinomyces* spp. with the use of PCR analysis appears to be extreme accurate. Amoxicillin should be considered as the first choice antibiotic for *Actinomyces* spp.

Key words: *Actinomyces*, dental caries, antibiotic, 16S rRNA.

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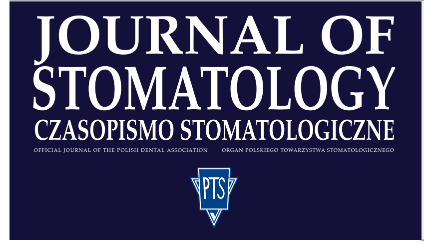
INTRODUCTION

Destruction of teeth is a serious problem in oral and general health of the human being, and dental caries is one of the major defects of dental hard tissue [1]. It is a chronic bacterial disease that affects dental structures caused by a huge number of bacterial communities that inhabit teeth surface, forming a biofilm of attached dental plaque. The first habitats are acidogenic bacteria, such as *Streptococcus mutans* and *Lactobacilli*, which find shelter in dental groves and fissures, and may be attached to smooth surface of inter-proximal areas as well as buccal and lingual regions. When optimum prevention methods are not followed, these biofilms are nourished by fermentable glucose and sugars derived from patient diet, forming a byproduct of lactic acids that causes dissolution of dental enamel, dentin, and even cementum by de-mineralization of these surfaces, followed by destruc-



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tion of the weakened surfaces by bacterial enzymes and toxins [2, 3]. It is a multi-episodic disease characterized by de-mineralization and re-mineralization from salivary buffering actions and minerals gained from food and prophylactic procedures [4]. The disease progresses when the compensating re-mineralization factors are no longer able to overcome destructive actions of the lesion microbiota [5]. Clinical feature of these invasions is the formation of white cavities and lesions that later undergo discoloration (brown or black). When getting deep into the tooth surface, this discoloration is caused due to staining from bacterial products or dietary origin [6], and when these lesions penetrate even deeper in dentine, pain and sensitivity are experienced by patients during consumption of sugary diet or drinking cold beverages. These symptoms can be more serious when the lesion is located closer to dental pulp or invade it causing severe pain and irreversible inflammation of the pulp. This condition may be acute or chronic, and if left untreated, it may cause the loss of pulp vitality and inflammation of the periapical and periodontal tissues with formation of abscess, while in cases of severe destruction, it may lead to loss of teeth and endangering para-oral structures and mid-facial spaces [7].

Studies on dental plaque reveal the presence of several species contributing to starting of adhesion and progression of the biofilm. The second largest contributing type of micro-organisms are anaerobic, Gram-positive (+ve) bacteria, called *Actinomyces* species [8-10], which have been demonstrated to be one of the pathogens to cause biofilms formation on tooth surfaces in patients of all ages [11]. Detection of *Actinomyces* species was also seen in root surface caries. Recent studies suggest that it has a major role in dental cavities with association of *Streptococci* and *Lactobacilli*, especially *Actinomyces naeslundii* [12]. The seriousness of actinomycotic infection gets more severe when these species penetrate into host tissues or skin causing a severe injury, called actinomycosis, that is a persistent infectious disease and its abortifacient agent, formerly known as *Streptothrix israeli*, have long been recognized [13].

The management of *Actinomyces* spp. usually entails prolonged courses of anti-microbials. Surgical intervention may also be necessary in advanced cases, depending on areas of infection (e.g., epidural infections, brain abscesses), in patients with large hemoptysis, or in cases where intensive abscesses and fistulous tracts are found [14]. *Actinomyces* spp. were found sensitive to a large variety of anti-microbial agents, such as penicillin, ampicillin, amoxicillin, and erythromycin [15, 16]. However, challenges with accurate *Actinomyces* genus identification and classification changes make it difficult to interpret multiple findings [17]. As our understanding of the illnesses caused by *Actinomyces* species advances, the knowledge of precisely recognized *Actinomyces* susceptibility may become increasingly significant [18]. For culturing of *Actinomyces* species, blood agar (BA) can be used with some nutritional requirements [19]. Gene sequencing based on the standard of 16S rRNA was introduced in the 80s of the past century, providing a reliable methodology for identification and isolation of studied bacterial species [20, 21], which is the methodology used in the current research. In order to identify *Actinomyces* spp., with quick and specifical isolation of these types of species in dental caries, and trying to investigate the effect of several antibiotic agents against them, the current study was conducted.

OBJECTIVES

The objective of this research was to isolate *Actinomyces* species from dental caries using polymerase chain reaction technology, and to examine the *in vitro* antibiotic resistance of isolates using a specific 16S rRNA primer to investigate the effect of several anti-bacterial agents, such as amikacin, gentamicin, ciprofloxacin, amoxicillin, ceftriaxone, cefixime, vancomycin, and erythromycin against *Actinomyces species*.

MATERIAL AND METHODS

SAMPLES COLLECTED AND METHODOLOGY

This cross-sectional survey study was conducted from March, 2021 to June, 2021 (three-month period). Eighty samples from patients with dental caries who visited a dental facility at the University of Babylon in Hilla City, Iraq, were collected and tested for the current investigation. Samples were obtained from each case by excavation of infected dentin from large carious lesions using a sterile spoon excavator. Each patient signed a consent form before being enrolled in the study and before sample collection. Samples were carefully gathered to prevent contamination. Part of the obtained specimens was instantly inoculated into a blood agar media at the patient's chairside. The remaining part of the sample was transferred to the Department of Microbiology for additional examination after being plated onto blood, MacConkey, mannitol, and nutrient agar media, and incubated for 24 hours at 37°C under aerobic and anaerobic conditions. Gram stain, colony morphology, and biochemical tests were performed to diagnose bacterial isolates. In addition, Vitek-2 Compact system (BioMérieux, Marcy-l'Étoile, France) was used for investigation of bacterial species with 16S rRNA technique. Each isolate underwent cultural, morphological, and biochemical testing to help with identification [22, 23]. *Actinomyces* spp. isolates were screened and identified by utilizing Vitek-2 Compact system. This form of identification is phenotypic, and relies on biochemical processes to identify the isolates. There were 64 wells on Vitek-2 card, each holding a separate fluorescent biochemical experiment. Phosphatase, urea, nitrate, and actidione assays were performed on 20 of

TABLE 1. Primer sequences for 16S rRNA genes with information and conditions on product size by base pair (bp)

16S rRNA genes	Primer sequence (5′-3′)	Size (bp)	Step No.	PCR conditions
Actinomyces meyeri	F: 5′-TCTGCGATTACTAGCGACTCC-3′ R: 5′-CCACCCGTGGTTTTCTGCG-3′	519	Step 1	95°C, 2 min
			Step 2	95°C, 30 sec.
			Step 3	60.3°C, 30 sec.
			Step 4	72°C, 60.0 sec.
			Step 5	Repeat steps 2-4 for 29 times
			Step 6	72°C, 5 min.
			Step 7	4°C, till end
Actinomyces viscosus	F: 5′-TCTGCGATTACTAGCGACTCC-3′ R: 5′-TCGTAGGCGGCTGGTCGC-3′	785	Step 1	95°C, 2 min.
			Step 2	95°C, 30 sec.
			Step 3	65.3°C decrease 0.5°C per cycle, 30 sec.
			Step 4	72°C, 80 sec.
			Step 5	Repeat steps 2-4 for 14 times
			Step 6	95°C, 30 sec.
			Step 7	58.3°C, 30 sec.
			Step 8	72°C, 80.0 sec.
			Step 9	Repeat steps 6-8 for 19 times
			Step 10	72°C, 5 min.
			Step 11	4°C, till end

the 64 samples for carbohydrate assimilation, followed by automatic filling and sealing of the cards; then, the cards were transported to incubator (35°C) and procedures were all handled by Vitek-2 machine. According to a specific algorithmic system, each output report was de-coded, and the acquired findings were identified by ID-GP (identification of Gram-positive bacteria data-bank). The corresponding supporting software automatically suggested ID results from these systems. When no ID or poor discrimination was indicated, the test was repeated and new results were considered in the analysis of data. After being plated into culture media, each strain was cultured at 37°C overnight, according to the manufacturer's instructions. Identification of *Actinomyces* spp. was done by specific 16S rRNA gene, as shown in Table 1, which presents the PCR settings and primer sequence utilized in the study [24].

EXTRACTING DNA FROM BACTERIAL ISOLATE

Using a genomic DNA purification kit and additional genomic DNA supplement, genomic DNA was isolated from each sample using Geneaid (Geneaid, USA) and observed with a UV-transmitting light source. Then, the sediments of bacterial cells were transported into a phosphate buffer solution and spun in micro-centrifuge at 14,000-16,000 rpm speed for one minute. Supernatant was then discarded with proteinase K, and 20 µl distilled water was added, followed by 10 minutes of incubation at 60°C, with three minutes interval of inversion. Actinomyces species were identified molecularly using the recovered DNA in a procedure described by the Institute of Clinical and Laboratory Standards [25].

TESTS TO DETERMINE ANTIBIOTIC SENSITIVITY (DISK DIFFUSION TEST) (TABLE 2)

The test was carried out using an isolated bacterial organism from a pure culture. Five isolated *Actinomyces* colonies were grown in blood agar mixed with 5 ml of nutrient broth, and used as an inoculum after incubation for 2 hours to produce a moderate turbidity bacterial suspension similar to cloudiness in ready-made McFarland tube standard. The inoculum was transmitted to a Muller-Hinton agar medium with sterile swabs. To find cells ex-

TABLE 2. Antibiotic disks used in the study

Company/origin	Potency	Antibiotic disks
Bioanalyse, Turkey	30	Amikacin (AK)
	10	Gentamicin (CN)
	5	Ciprofloxacin (CIP)
Biomaxima, USA	10	Amoxicillin (AX)
	30	Ceftriaxone (CRO)
	5	Cefixime (CFM)
	30	Vancomycin (Van)
	30	Erythromycin (E)

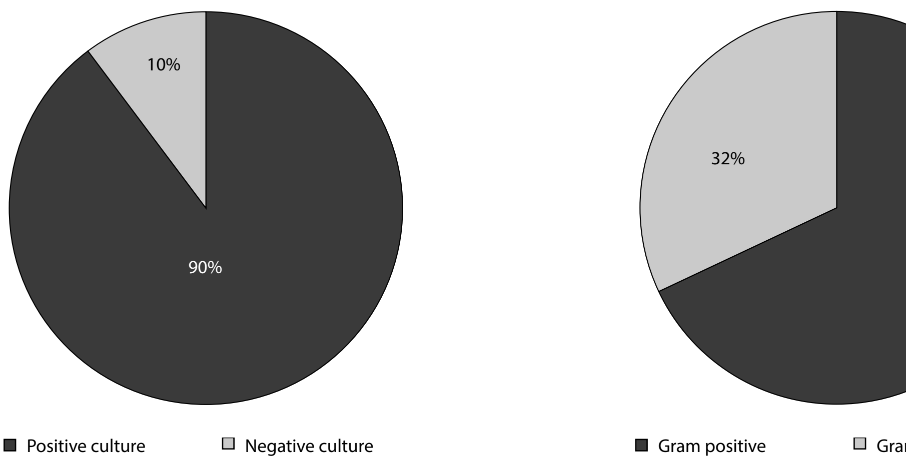

FIGURE 1. Positive and negative culture of all samples

FIGURE 2. Identification of bacteria according to Gram stain

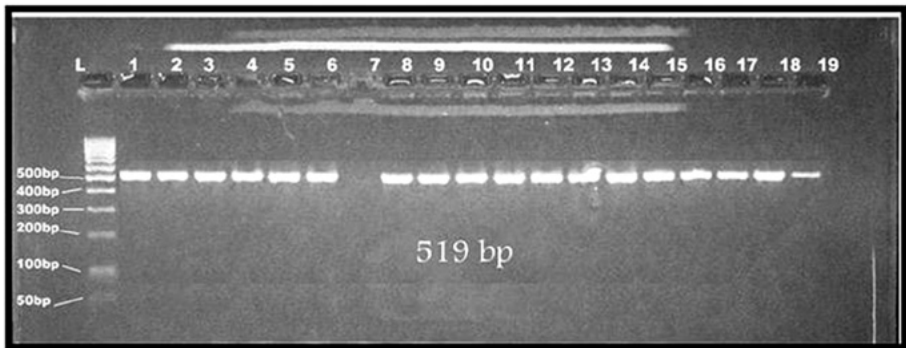

FIGURE 3. After being stained with ethidium bromide, 16S rRNA PCR products were electrophoretically separated on an agarose gel at 70 volts for 50 minutes. L: 1,500 bp ladder, lane; (1,2,3,4,5,6,8, … 19) were positive for *Actinomyces meyeri* gene; the size of product was 519 bp

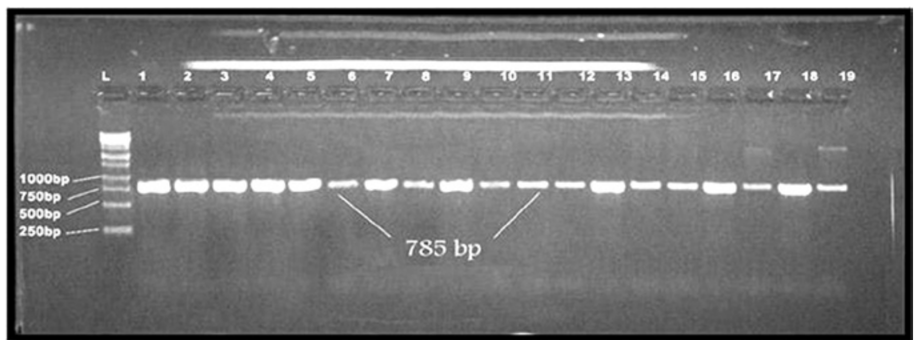

FIGURE 4. After being stained with ethidium bromide, 16S rRNA PCR products were electrophoretically separated on an agarose gel at 70 volts for 50 minutes. *Actinomyces viscosus* gene positivity in lanes 1-19 of a 1,500 bp ladder; the size of product was 519 bp

pressing hetero-resistance, antibiotic discs were placed onto these growth media using a flamed forceps, followed by incubation for 18 hours at 37°C. A transparent ruler was used to assess antibiotic inhibition areas, and the size of the zone was compared with the reference zone [25].

The technology of polymerase chain reaction (PCR) were applied to compare DNA strands with provided templates, and the specific sequence was multiplied into billions of copies (amplicon). In this study, *Actinomyces meyeri* and *A. viscosus* were detected by 16S rRNA genes, as shown in Table 1. All 18 (100%) *A. meyeri* samples identified with Vitek-2 system provided positive results for specific 16S rRNA gene at 519 bp (Figure 3), and all 19 (100%) *A. viscosus* samples identified by Vitek-2 system provided positive results for specific 16S rRNA gene at 787 bp, as shown in Figure 4.

All of the identified *A. meyeri* and *A. viscosus* were tested using disc diffusion method in Kirby-Bauer disc, to examine the in vitro antibiotic susceptibility of isolates that were isolated from dental caries using specific 16S rRNA primer. The utilization of selective antibiotic tests, such as amikacin, gentamicin, ciprofloxacin, amoxicillin, ceftriaxone, cefixime, vancomycin, and erythromycin demonstrated their impact on *A. meyeri* and *A. viscosus* isolates. The results corresponded to CLSI, 2023 [26], and are shown in Table 4 and Figures 5 and 6. Descriptive statistics were abbreviated, and the results were analyzed using SPSS software, version 19.0 (IBM Corp., Armonk, NY, USA).

RESULTS

After examining 80 specimens, 72 (90%) produced positive cultures, whereas 8 (10%) produced negative cultures (Figure 1, Table 3). The results showed that 44 (68%) out of 72 positive cultures on various growth media were categorized as Gram-positive, and 28 (32%) were Gram-negative (Figure 2, Table 3). In total, 18 (40.9%) bacterial isolates were associated with *A. meyeri*, while 19 (43.1%) isolates were related to *A. viscosus*, as shown in Table 3.

In all the isolates, antibiotic sensitivity of all the antibiotics employed in this study against *A. meyeri* was 18 (100%) for amoxicillin, 16 (88.8%) for ciprofloxacin, 14 (77.7%) for ceftriaxone, 13 (72.2%) for amikacin, 12 (66.6%) for gentamicin, 11 (61.11%) for cefixime, 7 (38.9%) for erythromycin, and 3 (16.6%) for vancomycin. Amoxicillin showed the greatest rate of antibiotic sensitivity compared with other antibiotics employed in this study against *A. meyeri*.

In all the isolates, antibiotic sensitivity of all the antibiotics employed in this study against *A. viscosus* was 19 (100%) for amoxicillin, 17 (89.4%) for ciprofloxacin, 14 (73.6%) for amikacin, 13 (68.4%) for ceftriaxone, 12 (63.15%) for gentamicin, 11 (57.8%) for cefixime, 7 (36.8%) for erythromycin, and 4 (21.02%) for vancomycin. The highest rate of antibiotic sensitivity was associated with amoxicillin among all the antibiotics employed in this study against *A. viscosus*.

DISCUSSION

Actinomyces species are available in different phenotypical and genotypical types. The cultivation of these types of species is considered difficult [27]. Furthermore, identification and differentiation of these anaerobic micro-organisms in clinical specimens are incredibly troublesome due to their complex transportation and growth requirements as well as their meticulous and delayed growth habits [28]. Initial efforts were to isolate *A. naeslundii* and *A. viscosus* types using catalase activity [29, 30]. The advancement of PCR technologies made it possible to identify different types of *Actinomyces* species, such as *A. bovis*, *A. viscosus*, *A. naeslundii*, *A. odontolyticus*, and *A. israeli* using 16S rRNA gene analysis procedure [31]. The development of molecular techniques corresponding to PCR and 16S rRNA improved the detection and distinction of closely related eubacteria species and enabled gene sequencing in a variety of ways [13]. A PCR methodology is extremely sensitive and may be adjusted to detect relatively few germs [32]. This ability of PCR is very helpful for distinguishing *Actinomyces* spp., since these organisms are

TABLE 3. Identification of *Actinomyces meyeri* and *Actinomyces viscosus* from other types of bacteria

Number of samples	Positive culture of other bacteria	Negative culture of bacteria	Gram-positive	Gram-negative	*Actinomyces meyeri*	*Actinomyces viscosus*
80	72 (90%)	8 (10%)	44 (68%)	28 (32%)	18 (40.9%)	19 (43.1%)
Total	80		72			

TABLE 4. Antibiotic susceptibility test of *Actinomyces meyeri* and *Actinomyces viscosus* isolates

No.	Antibiotic	Actinomyces meyeri		Actinomyces viscosus	
		Sensitive	Resistance	Sensitive	Resistance
1	Amikacin	13 (72.20%)	5 (27.80%)	14 (73.60%)	5 (26.30%)
2	Gentamicin	12 (66.60%)	6 (33.40%)	12 (63.15%)	7 (36.80%)
3	Ciprofloxacin	16 (88.80%)	2 (11.20%)	17 (89.40%)	2 (10.60%)
4	Amoxicillin	18 (100.00%)	0 (0.0%)	19 (100.00%)	0 (0.0%)
5	Ceftriaxone	14 (77.70%)	4 (22.30%)	13 (68.40%)	6 (31.50%)
6	Cefixime	11 (61.11%)	7 (38.90%)	11 (57.80%)	8 (42.10%)
7	Vancomycin	3 (16.60%)	15 (83.30%)	4 (21.05%)	15 (78.90%)
8	Erythromycin	7 (38.90%)	11 (61.11%)	7 (36.80%)	12 (63.15%)

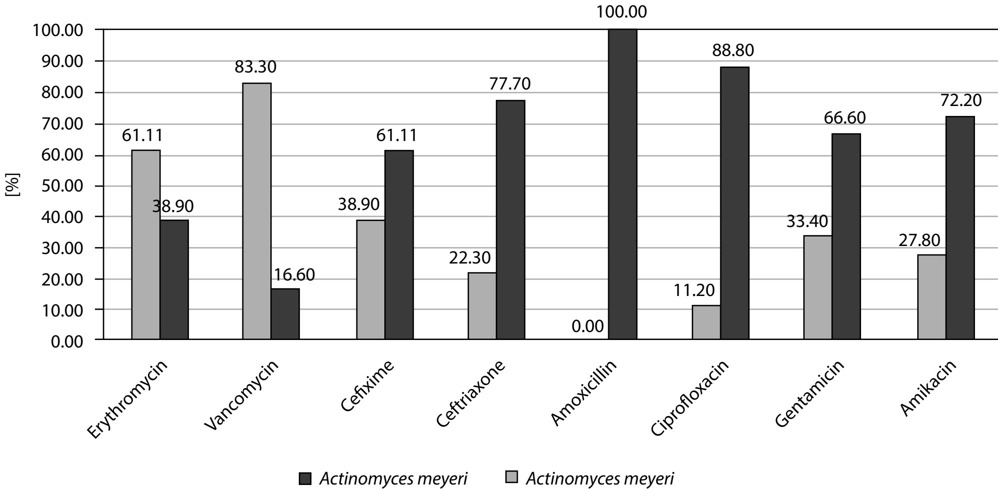

FIGURE 5. Antibiotic susceptibility test for *Actinomyces meyeri* isolates

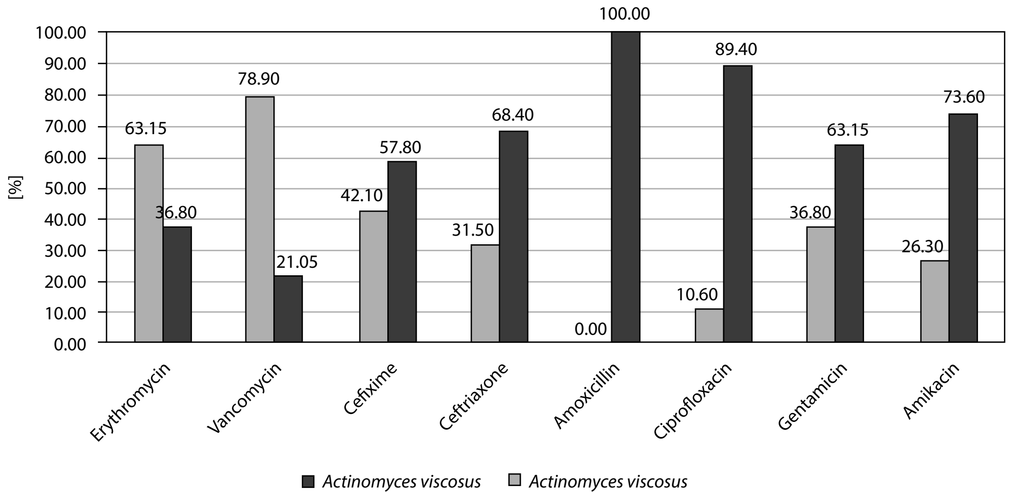

FIGURE 6. Antibiotic susceptibility test for *Actinomyces viscosus* isolates

available in low quantity in healthy individuals and not simply cultivated in wellness, which is attributable to their difficult and slow-growing nature [33]. Another characteristic of PCR is that it measures DNA rather than living organisms [34]. On the other hand, culture ways might not discover all micro-organisms, since they are not feasible and capable of growing into colonies [35]. Therefore, the diagnostic value of PCR is considered important once the micro-organisms are expected to be found in decay specimens [36]. The literature have investigated distinctly *Actinomyces* spp. In most studies on dental caries focused on complications related to these type of species in dental suppurative infections following some surgical procedures, it was confirmed that *A. meyeri* and *A. viscosus* may be found in dental caries [37]. Additionally, it was found to be part of the mixed flora in infections caused by *Eubacteria* species [38]. The aggregations of these types of micro-organisms are known clinically to form sulphur granules in abscesses and may complicate oral surgical treatments [39]. Because of this, current treatment plans include drugs that are effective against *Actinomyces*-associated lactamase producers, similar to *Staphylococci*, *Enterobacter*, and Gram-negative anaerobes [40].

The anti-microbial susceptibilities of numerous *Actinomyces* species include those of newer medications, such as augmentin [41]. A few individuals did not responded to amoxicillin at all or only in a limited degree, but they did responded favorably to long-term, high-dose treatment [42]. Rarely, treatment of chronic oral infections may last up to three weeks [43], strengthening the effect of amoxicillin using clavulanic acid. Also augmentin was found helpful, increasing the dosage of amoxicillin that is required in advanced chronic oral cases to increase tissue content of the drug, especially in mixed infections with enteric bacteria, such as *Enterobacter* spp. [44]. In oral actinomycosis, the latter is typically true. Addition of antiprotozoal or clindamycin for strict anaerobes may be required to fight more severe zoonosis illnesses [45]. When mixed with β-lactamase inhibitors, amoxicillin was

found to be powerful against *Actinomyces* spp. These data are in agreement with the results of a research [16]. In a clinical study, blood cultures and 16S rRNA sequencing techniques were applied to isolate *Actinomyces* species, specifically *A. viscosus*, from cellulitis infections that healed by intravenous administration of ampicillin. It was concluded that actinomycosis infections are easy to manage using a first-line treatment powered by penicillin and amoxicillin [46]. Additional data confirmed the effectiveness of penicillin [47]. In cases of bacteremia, subjects were treated by β-lactam antibiotics or cephalosporins in periods of 3 days to 6 months [48]. In antibiotic sensitivity tests, *Actinomyces* colonies were isolated from dental plaque samples adjacent to dental caries lesions with a percentage of 36.2%. All *Actinomyces* isolates were sensitive to penicillin and showed the highest inhibition among several types of antibiotics, with vancomycin reported as the lowest [12]. For patients allergic to aminopenicillins, tetracyclines and cephalosporins are suggested with a much lower clinical efficacy compared with penicillin and β-lactamase inhibitors [49].

CONCLUSIONS

The majority of antibiotics utilized in the current study against *A. meyeri* and *A. viscosus* showed a good rate of sensitivity. The highest rate of antibiotic sensitivity was associated with amoxicillin in all isolates, followed by ciprofloxacin, and then to a lesser degree, other antibiotics used in this study, including amikacin, ceftriaxone, gentamicin, cefixime, erythromycin, and vancomycin.

Moreover, the detection of *Actinomyces* spp. with PCR analysis appeared to be extreme accurate when used for presentation of antibiotics. Additionally, according to the findings of the current study, amoxicillin should be considered as the first choice antibiotic agent against *Actinomyces* species.

CONFLICT OF INTERESTS

The authors declare no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

References

1. Yadav K, Prakash S. Dental caries: a microbiological approach. J Clin Infect Dis Pract 2017; 2: 118.
2. Damé-Teixeira N, Parolo CCF, Maltz M. Specificities of caries on root surface. Monogr Oral Sci 2017; 26: 15-25.
3. Nayak M. Effect of modern (junk) food in dental caries. Indian Journal of Forensic Medicine and Toxicology 2020; 14: 9194-9197.
4. Khan S, Waliullah S, Godfrey V, et al. Dietary simple sugars alter microbial ecology in the gut and promote colitis in mice. Sci Transl Med 2020; 12: eaay6218. DOI: 10.1126/scitranslmed.aay6218.
5. Arifa MK, Ephraim R, Rajamani T. Recent advances in dental hard tissue remineralization: a review of literature. Int J Clin Pediatr Dent 2019; 12: 139-144.
6. Debbarma D, Mitra S. Occupational health problems of the auto rickshaws service providers in Agartala City: a case study of Nagerjala Motor Stand. Int J Curr Res Rev 2017; 9: 16-21.
7. Zajac J, Manrique Castrillon M, Fleury C, et al. Dental topics for plastic surgeons, part three: infection and trauma of the dentition. J Craniofac Surg 2021; 32: 805-812.
8. Brown JR. A study of morphology and microscopic examination of Actinomycetes from 181 subjects. Hum Pathol 1973; 4: 319-330.
9. Slots J, Möenbo D, Langebaek J, Frandsen A. Microbiota of gingivitis in man. Scand J Dent Res 1978; 86: 174-181.
10. Nyvad B, Kilian M. Microbiology of the early colonization of human enamel and root surfaces in vivo. Scand J Dent Res 1987; 95: 369-380.
11. Li J, Li Y, Zhou Y, Wang C, Wu B, Wan J. Actinomyces and alimentary tract diseases: a review of its biological functions and pathology. Biomed Res Int 2018; 2018: 3820215. DOI: 10.1155/2018/3820215.
12. Sivakumar T, Sukumar S, Yogananth N. Isolation and identification of dental caries causing Actinomycetes and antimicrobial activity of Punica granatum. Int J Curr Res Biol Med 2016; 1: 1-14.
13. Gajdács M, Urbán E. The pathogenic role of Actinomyces spp. and related organisms in genitourinary infections: discoveries in the new, modern diagnostic era. Antibiotics 2020; 9: 524. DOI: 10.3390/antibiotics9080524.
14. Holmgaard DB, Marina D, Hansen F, Christensen JJ. Bacteremia and urogenital infection with Actinomyces urogenitalis following prolonged urinary retention. APMIS 2020; 128: 20-24.
15. Barberis CM, Budia M, Palombarani S, et al. Antimicrobial susceptibility of clinical isolates of Actinomyces and related genera reveals an unusual clindamycin resistance among Actinomyces urogenitalis strains. J Glob Antimicrob Resist 2017; 8: 115-120.
16. Smith AJ, Hall V, Thakker B, Gemmell CG. Antimicrobial susceptibility testing of Actinomyces species with 12 antimicrobial agents. J Antimicrob Chemother 2005; 56: 407-409.
17. Shokeen B, Dinis M, Haghighi F, Tran N, Lux R. Omics and interspecies interaction. Periodontol 2000 2021; 85: 101-111.
18. Rahdar H, Mahmoudi S, Bahador A, Ghiasvand F, Heravi FS, Feizabadi MM. Molecular identification and antibiotic resistance pattern of actinomycetes isolates among immunocompromised patients in Iran, emerging of new infections. Sci Rep 2021; 11: 10745. DOI: 10.1038/s41598-021-90269-5.
19. Valour F, Senechal A, Dupieux C, et al. Actinomycosis: etiology, clinical features, diagnosis, treatment, and management. Infect Drug Resist 2014; 7: 183-197.
20. Woo PCY, Lau SKP, Teng JLL, Tse H, Yuen KY. Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. Clin Microbiol Infect 2008; 14: 908-934.
21. Fong P, Francis MJ, Hamblin JF, Korman TM, Graham M. Identification and diversity of Actinomyces species in a clinical microbiology laboratory in the MALDI-TOF MS era. Anaerobe 2018; 54: 151-158.
22. Baron EJ, Peterson LR, Finegoldens SM. Bailey and Scott's Diagnostic Microbiology. 9th ed. Mosology. Co., USA; 1994.
23. Mac Faddin JF. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Baltimore (Md.): Williams and Wilkins; 2000.
24. Lynch T, Gregson D, Church DL. Species-level identification of Actinomyces isolates causing invasive infections: multiyear comparison of Vitek MS (matrix-assisted laser desorption ionization-time of flight mass spectrometry) to partial sequencing of the 16S rRNA Gene. J Clin Microbiol 2016; 54: 712-727.
25. CLSI Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Approved standard M100-S17. 2019.
26. CLSI "Clinical and Laboratory Standards Institute". Performance standards for antimicrobial susceptibility testing. 2023. 33rd. Available from: https://clsi.org/standards/products/microbiology/documents/m100/ (Accessed: 24.04.2023).
27. Hall V, Brazier JS. Identification of actinomyces – what are the major problems? In: Eley AR, Bennett KW (eds.). Anaerobic Pathogens. Sheffield, UK: Academic Press; 1997, pp. 187-192.

28. Biswas S, Rolain JM. Use of MALDI-TOF mass spectrometry for identification of bacteria that are difficult to culture. J Microbiol Methods 2013; 92: 14-24.
29. Ellen RP, Balcerzak-Raczkowski IB. Differential medium for detecting dental plaque bacteria resembling Actinomyces viscosus and Actinomyces naeslundii. J Clin Microbiol 1975; 2: 305-310.
30. Schaal KP. Genus actinomyces. In: Sneath PHA, Mair NS, Sharpe ME, Holt JG (eds.). Bergey's Manual of Systematic Bacteriology. Williams and Wilkins: Baltimore, MD, USA; 1986, pp. 1383-1418.
31. Stackebrandt E, Charfreitag O. Partial 16 SrRNA primary structure of five Actinomyces species: phylogenetic implications and development of an Actinomyces israelii-specific oligonucleotide probe. J Gen Microbiol 1990; 136: 37-43.
32. Sambo F, Finotello F, Lavezzo E, et al. Optimizing PCR primers targeting the bacterial 16S ribosomal RNA gene. BMC Bioinformatics 2018; 19: 343. DOI: 10.1186/s12859-018-2360-6.
33. Buse HY, Morris BJ, Rice EW. Early detection of viable Francisella tularensis in environmental matrices by culture-based PCR. BMC Microbiol 2020; 20: 66. DOI: 10.1186/s12866-020-01748-0.
34. Gobert G, Cotillard A, Fourmestraux C, Pruvost L, Miguet J, Boyer M. Droplet digital PCR improves absolute quantification of viable lactic acid bacteria in faecal samples. J Microbiol Methods 2018; 148: 64-73.
35. Pearson B, Mills A, Tucker M, Gao S, McLandsborough L, He L. Rationalizing and advancing the 3-MPBA SERS sandwich assay for rapid detection of bacteria in environmental and food matrices. Food Microbiol 2017; 72: 89-97.
36. Lochman J, Zapletalova M, Poskerova H, Izakovicova Holla L, Borilova Linhartova P. Rapid multiplex real-time PCR method for the detection and quantification of selected cariogenic and periodontal bacteria. Diagnostics (Basel) 2019; 10: 8. DOI: 10.3390/diagnostics10010008.
37. Hug JJ, Bader CD, Remškar M, Cirnski K, Müller R. Concepts and methods to access novel antibiotics from Actinomycetes. Antibiotics (Basel) 2018; 7: 44. DOI: 10.3390/antibiotics7020044.
38. Stephenson T, Lee K, Griffith JE, et al. Pulmonary actinomycosis in South Australian koalas (Phascolarctos cinereus). Vet Pathol 2021; 58: 416-422.
39. Toprak N, Duman N, Sacak B, et al. Alloprevotella rava isolated from a mixed infection of an elderly patient with chronic mandibular osteomyelitis mimicking oral squamous cell carcinoma. New Microbes New Infect 2021; 42: 100880. DOI: 10.1016/j.nmni.2021.100880.
40. Nieder R, Benbi D, Reichl F. Soil as a transmitter of human pathogens. In: Soil Components and Human Health; 2018, pp. 723-827.
41. Woods GL, Brown-Elliott BA, Conville PS, et al. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes. 3rd ed.; 2019.
42. Savage RL, Tatley MV. Case series analysis of New Zealand reports of rapid intense potentiation of warfarin by roxithromycin. Drug Saf 2018; 41: 515-521.
43. Dym H, Zeidan J. Microbiology of acute and chronic osteomyelitis and antibiotic treatment. Dent Clin North Am 2017; 61: 271-282.
44. Ma YX, Wang CY, Li YY, et al. Considerations and caveats in combating ESKAPE pathogens against nosocomial infections. Adv Sci (Weinh) 2019; 7: 1901872. DOI: 10.1002/advs.201901872.
45. Teoh L, Cheung M, Dashper S, James R, McCullough M. Oral antibiotic for empirical management of acute dentoalveolar infections – a systematic review. Antibiotics (Basel) 2021; 10: 240. DOI: 10.3390/antibiotics10030240.
46. Hsiao YC, Lee YH, Ho CM, Tseng CH, Wang JH. Clinical characteristics of Actinomyces viscosus Bacteremia. Medicina 2021; 57: 1064. DOI: https://doi.org/10.3390/medicina57101064.
47. Wolff A, Rodloff AC, Vielkind P, Borgmann T, Stingu CS. Antimicrobial susceptibility of clinical oral isolates of Actinomyces spp. Microorganisms 2022; 10: 125. DOI: https://doi.org/10.3390/microorganisms10010125.
48. Maisa A, Almurtada R, Al-Maslamani M, Hamad AH. The spectrum and clinical significance of Actinomyces blood stream infection. Open Forum Infect Dis 2022; 9: 1861. DOI: 10.1093/ofid/ofac492.1490.
49. Dibek Misirlioglu E, Guvenir H, Ozkaya Parlakay A, et al. Incidence of antibiotic-related rash in children with Epstein-Barr virus infection and evaluation of the frequency of confirmed antibiotic hypersensitivity. Int Arch Allergy Immunol 2018; 176: 33-38.