

EFFICACY OF SODIUM HYPOCHLORITE 5.2% ON *ENTEROCOCCUS FAECALIS* AND IT'S DIAGNOSIS WITH ANTIBIOGRAM ISOLATED FROM INFECTED PULP

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Article Received on 27/05/2016

Article Revised on 18/06/2016

Article Accepted on 09/07/2016

ABSTRACT

Sodium hypochlorite has been used as an endodontic irrigant for more than 70 years, and is now one of the most common solutions for this purpose. Sodium hypochlorite is commonly used for irrigation of root canals in concentrations ranging from 1 to 5.2 %. This study purposed to investigate the antimicrobial efficacy of the Sodium hypochlorite NaOCl 5.2% on isolated *E. faecalis* from single rooted canals in Duhok city, Kurdistan province, Iraq. For this purpose two hundred extracted single rooted teeth were selected for this study. The root canals were prepared and shaped with sterile reamers (#15-55) and then the canal irrigation with sodium hypochlorite 5.2%, and microbiological samples were collected from each tooth using sterile paper points before and after the treatment with Sodium hypochlorite NaOCl 5.2% and inoculated into (brain heart infusion agar and blood agar). The bacterium was identified using colony morphology, Gram stain, biochemical tests and selective differential media. Further diagnosis confirmation and antibiotic susceptibility were done by BD Phoenix 100. Sixteen (8%) isolates of *Enterococcus faecalis* were isolated from 200 single rooted canals, after treatment with Sodium hypochlorite NaOCl 5.2% 11 (68.75%) isolates were eradicated, whereas only 5 (31.25%) isolate was resistant to treatment with Sodium hypochlorite NaOCl 5.2%. Before sodium hypochlorite 5.2% treatment, the percentage of *E. faecalis* isolates susceptible to nitrofurantoin was (93.75%), ampicillin and linezolid (65.25%), vancomycin and teicoplanin (50%). Resistant rate was observed with gentamicin (62.5%), but after sodium hypochlorite 5.2% treatment, the percentage of *E. faecalis* isolates susceptible to nitrofurantoin was (80%), and (60%) to the rest (gentamicin, ampicillin, teicoplanin, vancomycin and linezolid). This study highlighted that treatment with sodium hypochlorite 5.2% was effective in the elimination of *E. faecalis* isolated from infected rooted canals. And also BD phonix 100 system proved to be active in the identification and antibiotic susceptibility of *E. faecalis*.

KEYWORDS: *Enterococcus faecalis*, dental pulp, Sodium hypochlorite NaOCl 5.2%, BD phonix 100 system.**INTRODUCTION**

Enterococci are Gram-positive, facultative anaerobic, lactic acid bacteria, catalase negative. *Enterococci* occur as single cells, in pairs or as short chains, the optimum growth temperature is 35°C, but they can grow at temperatures between 10 - 45°C. *Enterococci* can even survive at 60°C for 30 minutes (Foulquie-Moreno *et al.*, 2006; Top *et al.*, 2008). They grow in broth containing 6.5% sodium chloride (NaCl), and hydrolyse esculin in the presence of 40% bile salts (Foulquie-Moreno *et al.*, 2006).

Enterococci are also able to survive extreme temperature and chemical disinfectants like chlorine, glutaraldehyde and alcohol (Bradley and Fraise, 1996). *Enterococcus faecalis* is a normal inhabitant of the oral cavity (Stuart *et al.*, 2006). *E. faecalis* is the most common *Enterococci* species, and it is responsible for 80-90% of human enterococcal infections (Jones *et al.*, 2004). *Enterococci* have also been reported as an important organisms in

periodontal infection (Peciuliene *et al.*, 2000). *Enterococci* are naturally resistant to antibiotics (Murray, 1990); they acquire antibiotic resistance and spread this to other species (Kuhn *et al.*, 2000).

Multiple antibiotic-resistant enterococci (MRE) emerge as a global threat to public health and threaten effective treatment of infections caused by the organisms (Rahangdale *et al.*, 2008). *E. faecalis* is resistant to many commonly used antimicrobial agents (Courvalin, 2006; Amyes, 2007). The most common reasons for failures in conservative root canal therapy are related to problems in instrumentation. However, occasionally, bacteria resistant to conservative therapy may also be involved (Siren *et al.*, 1997).

Virulence factors of *E. faecalis*, such as hemolysin, gelatinase, and enterococcal aggregation substance (EAS) play an important role in the bacterium's pathogenesis (Elsner *et al.*, 2000).

Increased antimicrobial resistance in enterococci has become a problem in recent years (Yong *et al.*, 2004). Vancomycin is often used as a drug of last resort in treatment of antibiotic-resistant, Gram-positive bacterial infections caused by organisms such as multi resistant enterococci, but the treatment is frequently unsuccessful (Dalal *et al.*, 2008; Sahlstrom *et al.*, 2009). *Enterococci* have proven to present a therapeutic challenge because of their resistance to many antimicrobial drugs "including cell-wall active agents; aminoglycosides, penicillin, ampicillin and vancomycin" (Paulsen *et al.*, 2003). Many studies have been directed towards finding an effective way to eradicate and/or prevent *E. faecalis* from gaining access to the root canal space. *E. faecalis* can gain entry into the root canal system during treatment, between appointments, or even after the treatment has been completed (Rocas *et al.*, 2004).

Sodium hypochlorite has been used as an endodontic irrigant for more than 70 years, and is now one of the most common solutions for this purpose (Clarkson and Moule, 1998). Sodium hypochlorite is both an oxidizing and hydrolyzing agent (Pashley *et al.*, 1985). Irrigation of root canals with sodium hypochlorite solutions (in concentrations ranging from 1 percent to 5.2 percent) is now a widely accepted technique. Sodium hypochlorite is commonly used for irrigation of root canals in concentrations ranging from 1 to 5.2 % (Young *et al.*, 2007).

The Phoenix Automated Microbiology System (BD Diagnostics System, Sparks, MD, USA) is designed for the rapid identification (ID) and antimicrobial susceptibility testing (AST) of clinically significant human bacterial pathogens (Fahr A.M. *et al.*, 2003). The testing process utilizes a duplex susceptibility test methodology using both a turbidity and oxidation-reduction indicator. These methods are employed in doubling antimicrobial concentrations which measures minimum inhibitory concentrations (MIC) at 20 minute intervals during the testing of panels (Fahr A.M. *et al.*, 2003). The Phoenix AST test is a modified miniaturized version of the micro-broth doubling dilution technique. Susceptibility testing in the Phoenix system is performed through determination of bacterial growth in the presence of various concentrations of the antimicrobial agent tested (Fahr A.M. *et al.*, 2003).

The study aims to

- 1-Investigate the antimicrobial efficacy of the sodium hypochlorite NaOCl 5.2% on isolated *E. faecalis* from single rooted canals.
- 2-Assess the antimicrobial activity of antibiotics for isolated *E. faecalis* by using BD phoenix 100 system.

MATERIALS AND METHODS

200 extracted teeth with single rooted canal were collected randomly from different private dental clinics in Duhok city, Kurdistan province, Iraq. The study was

carried out in the Department of Microbiology, School of Medicine, Faculty of Medical sciences, University of Duhok. The period of this study was from November 2010 to March 2011.

A reamers of suitable sizes (#15-55) were used to enlarge the canal and remove the pulp tissues. Instrumentation was followed by irrigation with normal saline. The initial microbiological samples were obtained by inserting sterile paper point into the prepared canal, then irrigation with sodium hypochlorite 5.2%, after that, adequate dryness was made for the canal, and a sterile paper point was used to obtain the second sample for microbiological isolation (Gomes, 2001).

The sterile paper points were cultured into brain heart infusion broth. After incubation at 37°C for 24 hours, each sample was sub-cultured on brain heart infusion agar, and blood agar. After incubation at 37°C for 24 hours, colony morphology were noted and diagnosed by catalase test and gram stain. The suspected colonies were sub-cultured onto bile esculin agar and incubated at 37°C for 24 hours, diagnosis and antibiotic susceptibility tests were done by BD Phoenix 100 for further confirmation.

Phoenix ID

The Phoenix system offers a combination ID and AST panel (PMIC/ID-70), with the identification substrates on one side and antimicrobial agents on the other side of the panel. The isolates were sub-cultured onto Bile Esculin Agar (BEA).

The Phoenix ID broth was inoculated with several bacterial colonies from a pure culture adjusted to 0.5 McFarland standard using a Phoenix Nephelometer (BD Diagnostics). After the transfer of 25 μ l of the ID broth suspension to the Phoenix AST broth, the remaining suspension was poured into the ID side of the panel.

Once the inoculated panel was labeled, it was logged and loaded into the instrument and incubated at 35°C. Purity plates were prepared for all isolates.

Phoenix AST

Preparation of the Phoenix AST broth requires adding a drop of Phoenix AST indicator (resazurin based dye acting as the terminal electron acceptor) before inoculation of 25 μ l of the broth from the standardized ID suspension. After addition of the ID broth suspension, the tube was mixed by inverting several times. The broth was then poured into the AST side of the panel. Once the inoculated panel was labeled, it was logged and loaded into the Phoenix apparatus and incubated at 35°C.

RESULTS

Among the 200 samples treated with sodium hypochlorite (5.2%), only 16 (8%) isolates of *E. faecalis* were isolated and 184 (89.5%) showed negative cultures for *E. faecalis*.

Table: 1 Number & percentage of *E. faecalis* response before & after treatment with NaOCl (5.2%).

No. of samples	positive isolates of <i>E. faecalis</i>	Negative isolates of <i>E. faecalis</i>	Before treatment with sodium hypochlorite	After treatment with sodium hypochlorite	
				Eradicated	Non eradicated
200	16 (8%)	184 (89.5%)	16 (100%)	11 (68.75%)	5 (31.25%)

11 (68.75%) isolates of *E. faecalis* were eradicated by sodium hypochlorite (5.2%) whereas only 5 (31.25%) isolates were resistant to sodium hypochlorite (5.2%) as shown in table (1).

Antibiotics Sensitivity of *E. faecalis* Isolates Obtained from Single Rooted Canals before Sodium Hypochlorite 5.2% Treatment

(2). Maximum susceptibility was observed with nitrofurantoin (93.75%), followed by ampicillin and linezolid (65.25%), vancomycin and teicoplanin (50%). Resistant rate was observed with gentamicin (62.5%).

Susceptibility to different antibiotics is shown in table

Table (2): Antibiogram of *E. faecalis* isolates obtained from single rooted canals before NaOCl (5.2%) treatment

Antibiotics	Susceptibility			Total No.
	Resistant (R)	Intermediate (I)	Sensitive (S)	
Gentamicin	10 (62.5%)	0%	6 (37.5%)	16
Ampicillin	7 (43.75%)	0%	9 (65.25%)	16
Teicoplanin	8 (50%)	0%	8 (50%)	16
Vancomycin	8 (50%)	0%	8 (50%)	16
Linezolid	7 (43.75%)	0%	9 (65.25%)	16
Nitrofurantoin	(6.25%)1	0%	15 (93.75%)	16

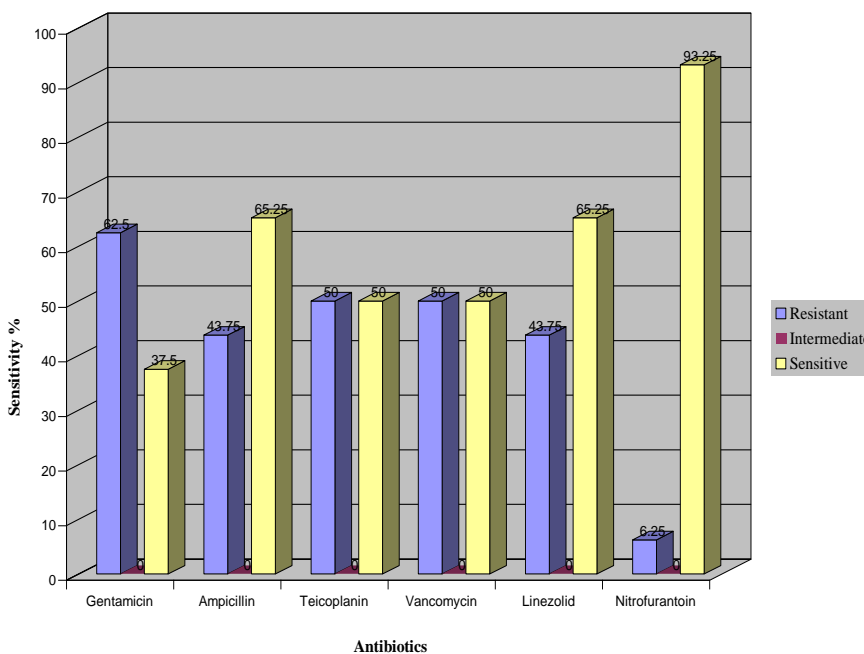


Figure (1): Antibiotics sensitivity of *E. faecalis* isolates obtained from single rooted canals before NaOCl (5.2%) treatment.

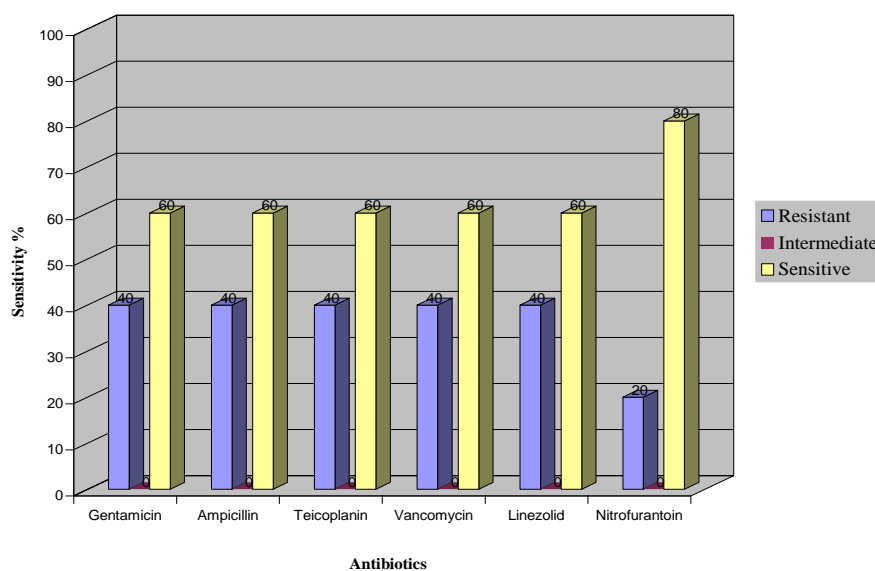
Antibiotics Sensitivity of *Enterococcus faecalis* Isolates Obtained from Single Rooted Canals after Sodium Hypochlorite 5.2% Treatment

(3). Maximum susceptibility was observed with nitrofurantoin (80%), followed by gentamicin, ampicillin, teicoplanin, vancomycin and linezolid (60%).

Susceptibility to different antibiotics is shown in table

Table (3): Antibiogram of *E. faecalis* isolates obtained from single rooted canals after NaOCl (5.2%) treatment.

Antibiotics	Susceptibility			Total No.
	Resistant (R)	Intermediate (I)	Sensitive (S)	
Gentamicin	2 (40%)	0%	3 (60%)	5
Ampicillin	2 (40%)	0%	3 (60%)	5
Teicoplanin	2 (40%)	0%	3 (60%)	5
Vancomycin	2 (40%)	0%	3 (60%)	5
Linezolid	2 (40%)	0%	3 (60%)	5
Nitrofurantoin	1 (20%)	0%	4 (80%)	5

**Figure (2): Antibiotics sensitivity of *E. faecalis* isolates obtained from single rooted canals after NaOCl (5.2%) treatment.**

DISCUSSION

Sodium hypochlorite is the most common irrigant used in endodontic practice, and the effectiveness of sodium hypochlorite has been confirmed by many studies (Gomes *et al.*, 2001; Radcliffe *et al.*, 2004; Zehnder, 2006; Mammani and Saleh 2010).

E. faecalis is the most resistant bacterial species to chemomechanical preparation, including instrumentation and irrigation with EDTA and NaOCl (Peciulienė *et al.*, 2001).

Our study showed that the treatment with sodium hypochlorite (5.2%) were eradicated 11(68.75%) isolates of *E. faecalis* out of 16 isolates.

Studies were evaluated the effectiveness of five concentrations of sodium hypochlorite (0.5%, 1%, 2.5%, and 5.25%) in the elimination of *E. faecalis* and showed that sodium hypochlorite at 5.25% was the most effective irrigants in eliminating the microorganism (Gomes *et al.* 2001; Berber *et al.*, 2006).

A study in Iraq demonstrated that sodium hypochlorite 5.2% was the most effective irrigant in the elimination of ampicillin 39 (92.9%), and 38 (90.5%) for gentamicin and vancomycin (Agarwal *et al.*, 2009).

E. faecalis in comparison to endox endodontic system (Mammani and Saleh 2010).

In the present study, Antibiotics sensitivity test was evaluated by using BD phoenix 100 apparatus.

In sodium hypochlorite (5.25%), before treatment *E. faecalis* isolates ($n=16$) were 93.75% sensitive for nitrofurantoin, 65.25% sensitive for ampicillin and linezolid, and 50% sensitive for teicoplanin and vancomycin. Gentamicin resistance percentage was 62.5%. While after sodium hypochlorite (5.25%) treatment, *E. faecalis* isolates ($n=5$) were 80% sensitive for nitrofurantoin, and 60% sensitive for gentamicin, ampicillin, teicoplanin, vancomycin, and linezolid.

A study in Italy showed that more than 90% of the isolates of *E. faecalis* were found susceptible to nitrofurantoin (Bonadio *et al.*, 2000). Similar susceptibility was found in the United States and Canada, which found that the *E. faecalis* were susceptible to linezolid and nitrofurantoin (Zhanel *et al.*, 2002).

An India study examined 42 *E. faecalis* isolates for susceptibility to antibiotics, they reported that all the isolates 42 were susceptible to teicoplanin, followed by

Also a study in Kuwait, observed that all the *E. faecalis* isolates (n=713) studied, were susceptible to teicoplanin, vancomycin and nitrofurantoin (Al Benwan *et al.*, 2010).

Other studies showed that all *E. faecalis* isolates were 100% sensitive to ampicillin (McGowan-Spicer *et al.*, 2008; Valenzuela *et al.*, 2008& 2009).

CONCLUSION

1-We concluded that the treatment with sodium hypochlorite 5.2% was effective in the elimination of *E. faecalis* isolated from infected rooted canals.

2-BD phoenix 100 system proved to be active in the identification and Antibiotic susceptibility of *E. faecalis* than the traditional methods (Culturing methods).

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