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RESEARCH ARTICLE

MICROBIOLOGICAL CONTAMINATION OF NORMAL SALINE SOLUTION USED DURING ENDODONTIC TREATMENT AT DIFFERENT TIME INTERVALS- AN *IN VITRO* STUDY

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ABSTRACT

Aim: To examine possible microbiological contamination of normal saline solution under normal clinical surroundings, also to determine the duration for which dentist could use their normal saline solution without microbiological contamination using different techniques and to identify common micro organisms that may contaminate such solutions.

Materials and Methods: Three procedures for collecting samples of the normal saline from each of three sealed plastic bottles available in market were used comparable to procedure routinely followed in clinics.

1. The bottle 1 was cut with sterile B.P. blade and normal saline solution was taken into a disposable plastic glass.
2. Evacuate normal saline solution from bottle 2 using sterile syringe
3. Evacuate normal saline from bottle 3 using sterile syringe and solution was taken into sterile autoclave container with all aseptic precautions.

Same procedure was followed for each of three normal saline bottles of brand two.

Samples were collected at 0, 2, 4 hours of clinical work timings every day for three consecutive days. Every time immediately after collecting sample of saline, it was inoculated onto the blood agar and MacConkey agar. Plates are then incubated. After obtaining growth, gram staining was done to identify and categorize the bacteria.

Results: On day 1 of study, growth starts to appear in disposable glass technique by the end of day i.e. at 4 hr interval sample which consisted of gram positive cocci. Growth in the case of sterile syringe method started to appear at 2 hrs on day 3 which was also gram positive cocci and in the case of sterile container growth started to appear at 4 hr sample on day 3, again gram positive cocci.

Conclusions: The present study showed that microbiological contamination does occur whatever technique may be followed. Saline from sealed bottle should be used for once, then it should be discarded. However, amongst methods used in study, the best method that dentist could use is the use of sterile autoclaved container which can be best used maximum for two days using same bottle of saline. The use of disposable glass for keeping normal saline solution to be used root canal irrigation should be discarded. Also, this study indicated that most common contaminant in this solution was gram positive cocci.

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INTRODUCTION

Elimination or significant reduction of irritants and prevention of re contamination of the root canal after treatment are the essential elements for successful outcomes of root canal treatment. (Azhar Iqbal 2012; American association of endodontists; WINTER 2011) The major cause of pulpal and periapical diseases are non living and living irritants. Non living ones can be mechanical, thermal or chemical irritants. The living ones include various micro-organisms including bacteria, yeast and viruses. (American association of endodontists, Winter 2011) Chemomechanical root canal

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preparation is capable of reducing the number of micro-organisms in the system, but achievement of complete disinfection is difficult due to the complex anatomy of the canal system that includes presence of numerous dentinal tubules, lateral and accessory canals and their invasion by micro-organisms. (Aranda-Garcia *et al*., 2012) Several irrigating solutions have been used to flush out irritants from root canals (Aranda-Garcia *et al*., 2012). All irrigating solutions must be sterile as the prepared root canal is prone to infection if irrigated with a contaminated solution. Among them, 0.9% normal saline is very popular and commonly used conventional intracanal irrigant. (Paudel *et al*., 2011) Normal saline has no antibacterial action, but has only the flushing action during intracanal irrigation. (Wilson *et al*., 1990) Unfortunately, while treating a patient for disease, there is sometimes risk of developing health care associated infection (HAI). Any HAI has an impact on the health outcome of a patient which in turn results in a financial burden on a patient as well as the clinic, as these patients have to come for repeated visits. (Amy S. Collins) In a case of root canal treatment, although we are using sterile normal saline solution for irrigation but it has been a common practice among practitioners to use a bottle of normal saline for some days until it gets finished, perhaps owing to economic reasons. But micro-organisms can grow in these open normal saline bottles. So, this microbiological study was conducted to examine (a) possible microbiological contamination of normal saline under normal clinical surroundings. (b) to determine the duration for which dentist could use their normal saline solution without microbiological contamination by using different techniques. (c) to identify common micro-organisms that may contaminate such solutions. (d) and to identify the best method of using normal saline solution as root canal irrigant in dental clinics.

MATERIALS AND METHODS

Three procedures for collecting the normal saline from sealed plastic bottles available in market were used comparable to procedure routinely followed in clinics.

Two brands of preservative free normal saline solution (Brand one:NS 0.9% Sodium chloride injection I.P., Pentagon labs limited, India and Brand two: Albert David Limited) commercially available and commonly used were evaluated in this study. So, six new normal saline bottles (three bottles of each brand) were taken for study.

Study on three normal saline bottles of brand one was done as under

1. Bottle 1 was cut with sterile B.P. blade and solution was taken into disposable plastic glass.
2. Solution was evacuated from bottle 2 using sterile syringe
3. Solution was evacuated from bottle 3 using sterile syringe and solution was taken into sterile autoclaved container with all aseptic precautions.

Study on three normal saline bottles of brand two was done following same procedure as for brand one. On day one, using each bottle the first sample was collected at the start of day which will serve as starting first sample and control as well to check if bottle was not previously contaminated. This was indicated as sample taken at 0 hrs. After these samples from bottles were taken at 2, 4 hours corresponding to 6 hours of clinical work timings. Every time immediately after collection, samples of saline solution were inoculated on the blood agar and MacConkey agar. Plates are then incubated for 24 hours at 37 degree Celsius. After the growth was obtained, gram staining was done to identify and categorize the bacteria into gram positive/ gram negative or cocci/bacilli. On day 2 and 3 same procedure was followed and results compiled as indicated in Table 1 and Table 2.

RESULTS

Growth results of the different samples of normal saline solution collected using different techniques were compiled in tables below which include the comparison of all three methods repeated on three consecutive days.

BRAND ONE (Table 1)

Days	Time (in hours)			
		Disposable glass	**Sterile syringe**	**Sterile container**
Day 1	**0**	No growth	No growth	No growth
	2	No growth	No growth	No growth
	4	Growth present (gram positive cocci)	No growth	No growth
Day 2	**0**	Growth present(gram positive cocci and bacilli)	No growth	No growth
	2	Growth present(gram positive cocci and bacilli)	No growth	No growth
	4	Growth present(gram positive cocci and bacilli)	No growth	No growth
Day 3	**0**	Growth present (gram positive cocci and bacilli)	No growth	No growth
	2	Growth present(gram positive bacilli and cocci and gram negative bacilli)	Growth present (gram positive cocci)	No growth
	4	Growth present(gram positive bacilli and cocci and gram negative bacilli)	Growth present (gram positive cocci)	Growth present (gram positive cocci)

BRAND TWO (Table 2)

Days	Time (in hours)			
		Disposable glass	**Sterile syringe**	**Sterile container**
Day 1	**0**	No growth	No growth	No growth
	2	No growth	No growth	No growth
	4	Growth present (gram positive cocci)	No growth	No growth
Day 2	**0**	Growth present(gram positive cocci and bacilli)	No growth	No growth
	2	Growth present(gram positive cocci and bacilli)	No growth	No growth
	4	Growth present(gram positive cocci and bacilli)	No growth	No growth
Day 3	**0**	Growth present (gram positive cocci and bacilli)	Growth present (gram positive cocci)	No growth
	2	Growth present(gram positive bacilli and cocci and gram negative bacilli)	Growth present (gram positive cocci)	Growth present (gram positive cocci)
	4	Growth present(gram positive bacilli and cocci and gram negative bacilli)	Growth present (gram positive cocci)	Growth present (gram positive cocci)

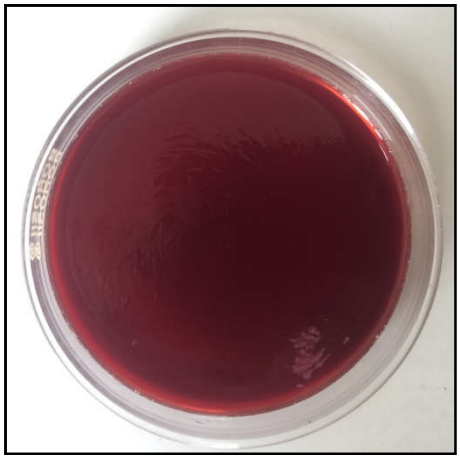

Fig. 1. Growth of cocci on blood agar

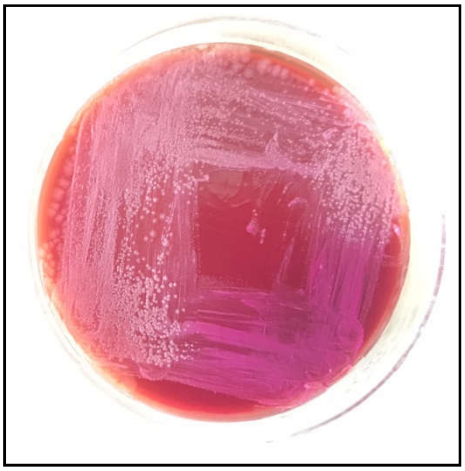

Fig. 2. Mixed growth on MacConkey agar

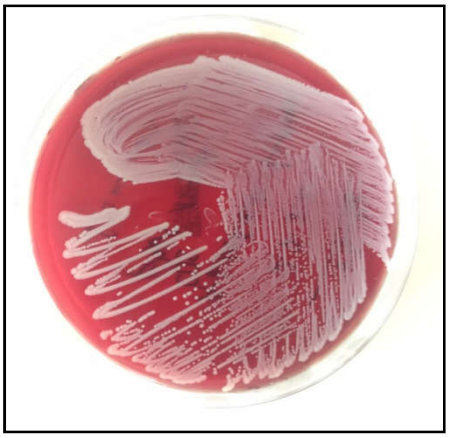

Fig. 3. Mixed growth on blood agar

From results, we can conclude that on day 1 of study, growth started to appear in disposable glass technique by the end of day i.e. at 4 hr interval sample which consisted of gram positive cocci, whereas in other two techniques i.e. use of sterile syringe and sterile container did not show any growth on day 1 of the study. On day 2 of this study, same bottles were continued for sterile syringe and sterile container method. However, use of disposable glass bottle was discontinued as it had started to show positive growth. But that bottle was not discarded and repeated cultures from same bottle were still taken put up to find any more microorganisms if it adds up. Same procedure was repeated on day 3. It was found on day 3 that all the procedures had shown growth so the bottles were discarded. Growth in case of sterile syringe method started to appear on day 3 which was gram positive cocci and in case of sterile autoclaved container growth started to appear at 4 hr sample (brand 1) and 2hr sample (brand 2) on day 3, again gram positive cocci. Growth of gram positive bacilli were found on day 2 and gram negatve bacilli on day 3 in 2 hr sample in disposable glass method.

DISCUSSION

Isotonic sterile normal saline is the most common solution used for irrigation of root canal. (Engle, 1993) Sodium chloride is a white, crystalline powder or colorless crystals, freely soluble in water and practically insoluble in ethanol. Sodium chloride injection solution is sterile, isotonic, preservative free solution containing sodium chloride BP 0.9% in water. There is a substantial amount of evidence to suggest that improperly sterilized products can severely harm patients. (Amy S. Collins) Point to be considered is that degree of microbial viability varies from one solution to other. In this study, normal saline solution was used which show no antimicrobial activity. Present study showed growth of bacteria in method using disposable glass on day 1, indicating that this method is bound to give infection to the patient if used. As compared to above method, other two methods i.e. sterile syringe and sterile container showed growth on third day. Growth was same as on day 1 in disposable glass i.e. gram positive cocci. This indicates that gram positive cocci are the most common contaminant pathogen present in our setting and that it can cause opportunistic pathogenesis of bottle containing normal saline solution if used for prolonged time. Findings in this study suggest that nutritional parental products tend to be uniformly vulnerable to micro organism proliferation upon contamination. Normal saline do not have any significant inhibitory effect on many colonizing bacteria. (Rawal *et al*., 1985) However some of the other studies (Houang *et al*., 2001, Ephigenia *et al*., 2003) have reported that gram-negative bacteria are the predominant microbes found in unpreserved saline while gram-positive bacteria are more often found in preserved saline (Sweeney *et al*.,1999). Authors believed that practice of hygiene in handling of saline solution is important factor to reduce contamination. This emphasizes that care should be observed when using the normal saline to prevent contamination of nozzle or any part that is in contact with the normal saline, which may lead to contamination of the content in the bottle. (Chua Siew Siang *et al*., 2006) This normal saline solution is suitable growth substrate for micro-organisms once introduced during improper handling. The susceptibility of the solution should be taken into account to decrease the likelihood that a contaminating microbe could proliferate to increasingly dangerous levels. We can potentially decrease the overall risk of infection during root canal treatment procedure by using saline solution in a proper way, thereby improving patient outcomes and decreasing overall medical expenditures. One of the limitations in this study is that the microbial contamination detected could be from the environment, introduced into the sample during collection or specimen handling in the clinics. In

addition, the microorganisms detected could be present only on the orifice of the dispenser tip of the normal saline bottles and may not necessarily show that the bottle content was contaminated (Wilson *et al*., 1990). Another unavoidable limitation is that, if a minute number of microorganisms is present in the normal saline these may not be sampled. A more accurate method of evaluating the presence of microorganisms is to filter the whole content of normal saline and then to culture the filter. This is not possible as the aim of this study is to determine whether the use of normal saline can be extended up to some days, therefore, the filtration method would render the solution not usable after each sampling. The brand of normal saline used in the present study had no effect on the contamination rate. This may be because the two brands tested in this study are of similar quality and they both contain only 0.9% sodium chloride, without any preservative. The duration of use has a role to play, but the place where the normal saline was used was not related to the incidence of microbial contamination as study on both bottles was conducted at same place. Some people prepare their own preservative free saline using salt tablets and USP purified water (Engle, J. P. 1993). Ideally fresh bottle of saline solution or freshly prepared solution should be used and practice of using same saline bottle for many days from which aliquots are withdrawn should be abandoned. Although not recommended, if in case normal saline bottle is used again ,then sterile syringe should be used to evacuate the saline and disposed in autoclaved container instead of disposable glass. Bottle of saline solution should not be used for more than two days.

Conclusion

This study shows growth of bacteria in normal saline solution used as endodontic irrigant if used for prolonged period irrespective of method used. Ideally bottle containing sterile solution should be used once or every time freshly prepared solution should be made using sterile components,

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