ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 02, pp.26557-26562, February, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

A COMPARATIVE STUDY FOR EFFICACY OF POVIDONE IODINE IN COMBINATION WITH ORNIDAZOLE AND METRONIDAZOLE IN ANAEROBIC ISOLATES

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ARTICLE INFO

Article History:
Received 20th November, 2015
Received in revised form
08th December, 2015
Accepted 24th January, 2016
Published online 27th February, 2016

Key words:

Anaerobes,
Metronidazole,
Ornidazole,
Povidone iodine,
Wound infection.

ABSTRACT

Background: The majority of infected wounds are polymicrobial and their treatment is complicated owing to relatively slow growth of organisms and growing resistance of anaerobic bacteria to antimicrobial agents. Hence, the present study was initiated to assess the efficacy of Povidone iodine alone and in combination with Ornidazole and Metronidazole in anaerobic isolates.

Methods: Brucella blood agar plates were inoculated with suspension of anaerobic clinical isolates of *Peptostreptococcus magnus, Clostridium perfringens,* and *Bacteroides fragilis.* After solidification, 100 µL drug solutions of each antimicrobial agent were added, incubated in anaerobic conditions. Zone of inhibitions were measured after 7-9 days to determine minimum inhibitory concentration.

Results: Combination of Povidone iodine 5% and Ornidazole 1% was the most effective combination in inhibiting the growth of *Bacteroides fragilis and Clostridium perfringens* whereas the combination of Povidone iodine 5% and Metronidazole 1% was more effective in inhibiting the growth of *Peptostreptococcus magnus* than Povidone iodine alone / other drug combinations.

Conclusion: The present study reveals that addition of Ornidazole or Metronidazole to Povidone iodine was more effective, than Povidone iodine alone, in inhibiting the growth of all tested anaerobic bacteria namely *Bacteroides fragilis, Clostridium perfringens, and Peptostreptococcus magnus*.

Citation: **Tejal Vedak, Namrata Kulkarni, Vandit Trivedi, Bhasker Vyas, Satessh Siingh, Rajendra Musale and Mangesh Bhalerao, 2016.** "A comparative study for efficacy of Povidone Iodine in combination with Ornidazole and Metronidazole in anaerobic isolates", *International Journal of Current Research,* 8, (01), 26557-26562.

INTRODUCTION

Microbial colonization proliferates in a wound on exposure to moist, warm or nutritious environment. Since wound colonization is most frequently polymicrobial, (Bowler, 1998; Bowler and Davies, 1999) involving numerous microorganisms that are potentially pathogenic, any wound is at some risk of becoming infected. To date, widespread opinion among wound care practitioners is that aerobic or facultative pathogens such as *Staphylococcus aureus, Pseudomonas aeruginosa*, and *β-hemolytic streptococci* are the primary causes of delayed healing and infection in both acute and chronic wounds. (Daltrey *et al*., 1981; Gilliland *et al*., 1988; Twum-Danso *et al*., 1992) Since, infection is often polymicrobial and is caused by aerobic and anaerobic organisms; antimicrobials that are effective against both components of the infection should be taken into consideration in the course of its treatment. Infections caused by anaerobic bacteria are common and may be serious and life-threatening. Anaerobes are the predominant components of the bacterial flora of normal human skin and mucous membranes. (Hentges, 1993) They are the most common cause of bacterial infections of endogenous origin. Because of their fastidious nature, they are difficult to isolate from infectious sites and are often overlooked. (Brook, 2007; Finegold, 1977; Jousimies-Somer *et al*., 2002) Treatment of such anaerobic bacterial infections is very complicated. A review of the literature indicates that anaerobic bacteria constitute, on an average, one-third of the total number of microbial species in colonized wounds, and this number increases to approximately 50% in the infected wounds. (Nagy, 2010; Bowler *et al*., 2001) Therefore, antimicrobial treatment of clinically infected and/or non-healing polymicrobial wounds should cover a variety of potentially synergistic aerobic and anaerobic microorganisms. The commonly used antiseptic agent for wound management is Povidone iodine, which is an

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iodophore antiseptic and is a broad spectrum microbicidal drug. It has a broad antimicrobial spectrum: bacteria, viruses, bacterial endospores, fungi, and protozoans are destroyed, however, been limited by a number of undesirable factors. Several in vitro studies have reported the efficacy Nitroimidazole, including ornidazole and metronidazole, with significant activity against anaerobic microorganisms. Ornidazole has been found to be a promising substance for the therapy of anaerobic infections as the in vitro inhibitory activity of Ornidazole was determined against 150 isolates of clinically important anaerobes including *Bacteroides fragilis, Bacteroides bivius* and *Clostridium perfringens.* Similarly, efficacy of metronidazole against obligate anaerobic bacteria including the gram- negative organisms (*Bacteroides fragilis, Fusobacterium Spp., Peptococcus Spp, Peptostreptococcus spp. and Villanelle Spp.)* is well established. As it is ineffective against aerobic bacteria, for treatment of polymicrobial infection, Ornidazole and metronidazole should be used in combination with other antibacterial agents that are appropriate for the treatment of the aerobic infection. (Nichols and Smith, 1994) As there is dearth of knowledge to show the efficacy of Povidone iodine in combination with Ornidazole and Metronidazolein anaerobic isolates, the present study was conducted to evaluate the synergistic action of Povidone iodine and nitroimidazole derivatives. The objective of present study was to evaluate the efficacy of Povidone iodine alone and in combination with Ornidazole and Metronidazole in anaerobic clinical isolates of *Bacteroides fragilis, Clostridium perfringens* and *Peptostreptococcus magnus.*

MATERIALS AND METHODS

In the present study, four different drugs and drug combinations were used; viz; i) Povidone iodine 5%, ii) Povidone iodine 10%, iii) Povidone iodine 5% in combination with Ornidazole 1%, and iv) Povidone iodine 5% in combination with Metronidazole 1%. All the drugs were procured and supplied by RPG Life Sciences Ltd. Materials used for microbiology analysis and procedures performed in this study were according to standard operating procedures (SOP) of APL Institute of Clinical Laboratory and Research Pvt. Ltd, Ahmedabad, India. Glassware, glass pipettes, micropipette tips, and stainless steel hollow cylinder (6mm diameter) were sterilized in oxygen free environment prior to use.

Microbial Cultures

For the present study, clinical isolates of *Bacteroides fragilis, Clostridium perfringens,* and *Peptostreptococcus magnus* were procured from Lal Pathlabs, Delhi, India. These stock culture suspensions were stored and maintained at APL Institute of Clinical Laboratory and Research Pvt. Ltd, Ahmedabad, India, at 2-8 °C before use.

Working culture suspensions of all bacteria were prepared in 0.45% saline under oxygen free environment, from the stock suspension just before use. The density of the bacterial colonies in the working culture, as measured by Densitometer (AK4286; BioMérieux, USA), was equal to 0.5 McFarland standard (mcf).

Culture Medium

Ready to use modified Brucella Blood Agar (BRU) plates were procured from BD Biosciences, Chennai, India (Batch no: 5041720). BD Brucella Blood Agar with Hemin and Vitamin K1 is a modification of Brucella Agar that has been supplemented with hemin and vitamin K1 to support the growth of fastidious anaerobes, especially Bacteroides, when incubated anaerobically. The advantages of ready to use BD Brucella Blood Agar plates are uniformity in the content of the medium throughout the experiment and uniform thickness of the medium. The size of the plates used was 100 × 15 mm for all experiments. The composition of medium is described in Table I.

Drug Dilutions

Serial dilutions of each drug/drug combination were prepared using sterile distilled water to determine zone of inhibitions and MIC. The serial dilutions used for first set of experiments were: Neat, 1:2, 1:4, 1:10, 1:50, 1:100, 1:200, 1:500, and 1:1000. Additionally, for Povidone iodine 5% with Ornidazole 1% for *Bacteroidesfragilis*, dilutions of 1:2000, 1:3000, 1:4000 and 1:5000 were also prepared as zone of inhibitions initially obtained with above mentioned dilutions were higher. After the results of first set of experiments, for those dilutions whose zone of inhibitions were higher than 12 mm, additional dilutions were prepared in second set of experiments for precise determination of zone of inhibition. Table II describes specific dilutions used in second set of experiments.

Inoculation

Brucella blood agar plates were inoculated with 0.5 mcf (equivalent to 1.5×10^8 CFU/ml) bacterial suspension in an anaerobic environment at 35-37 °C and the plates were rotated through an angle of 60° to obtain uniform growth of microorganisms. Inoculum was left to dry and absorbed for few minutes with lid closed at room temperature. Then with the help of sterile stainless steel hollow cylinder, 6mm wells were made in the plates. 100 µL drug solutions of each dilution of two different Povidone iodine concentrations (5% & 10%) and its combinations with either Metronidazole (1%) or Ornidazole (1%) were then poured in these wells using micropipette. The plates were then incubated in anaerobic jar (Hi-Media laboratories) for 7-9 days until clear zone of inhibitions were observed. This was confirmed by periodic checking at every 24 hrs.

Determination of Zone of Inhibition and Minimum Inhibitory Concentration (MIC)

All the experiments were done in triplicates and the mean of three readings was calculated. Zone of inhibition was measured using Zone Scale reader (Hi-Media laboratories) and noted in millimeter. The drug dilution which gives the smallest zone of inhibition was considered as MIC.

RESULTS

Serial dilutions of various drug concentrations gave different zones of inhibition in first set of experiments.

Table 1. Composition of Brucella Blood Agar (BRU)

Item	Quantity (g/l of purified water)
Pancreatic Digest of Casein	10.0 g
Peptic Digest of Animal Tissue	10.0
Yeast Extract	2.0
Glucose	1.0
Sodium Chloride	5.0
Sodium Bisulfite	0.1
Hemin	0.005
Vitamin K1	0.01
Agar	15.0
Sheep Blood, defibrinated	5%

Table 2. Additional Drug Dilutions

Microorganism	Drug	Dilution
Bacteroides fragilis	Povidone iodine 5%	1:20
	Povidone iodine 5% + Metronidazole 1%	1:150
	Povidone iodine 5% + Ornidazole 1%	1:1500
Clostridium perfringens	Povidone iodine 5%	1:75
	Povidone iodine 10%	1:75
	Povidone iodine 5% + Metronidazole 1%	1:75
	Povidone iodine 5% + Ornidazole 1%	1:150
Peptostreptococcus magnus	Povidone iodine 5%	1:350
	Povidone iodine 10%	1:350
	Povidone iodine 5% + Metronidazole 1%	1:800
	Povidone iodine 5% + Ornidazole 1%	1:350

Table 3. Zone of Inhibition of Different Drug Dilutions

Microorganism	Drug	Zone of Inhibition (mm)																		
		Dilution																		
		Neat	1:2	1:4	1:10	1:20	1:50	1:75	1:100	1:150	1:200	1:350	1:500	1:800	1:1000	1:1500	1:2000	1:3000	1:4000	1:5000
Bacteroides fragilis	Povidone iodine 5%	>40	22	18	14	<6	<6		0		0		0		0					
	Povidone iodine 10%	>40	30	25	22		19		10		<6		0		0					
	Povidone iodine 5% + Metronidazole 1%	>40	27	23	20		17		13	<6	<6		0		0					
	Povidone iodine 5% + Ornidazole 1%	36	33	31	29		27		24		21		18		16	15	<6	0	0	0
Clostridium perfringens	Povidone iodine 5%	34	23	20	18		16	<6	<6		0		0		0					
	Povidone iodine 10%	38	24	22	19		17	<6	<6		0		0		0					
	Povidone iodine 5% + Metronidazole 1%	36	24	20	18		16	<6	0		0		0		0					
	Povidone iodine 5% + Ornidazole 1%	>40	25	21	19		17		15	<6	<6		0		0					
Peptostreptococcus magnus	Povidone iodine 5%	>40	35	32	28		23		18		12	<6	<6		0					
	Povidone iodine 10%	>40	36	34	31		28		21		15	<6	<6		0					
	Povidone iodine 5% + Metronidazole 1%	>40	34	30	27		22		16		13		11	<6	0					
	Povidone iodine 5% + Ornidazole 1%	>40	32	29	25		22		18		13	<6	<6		0					

Table 4. MIC of Different Drug Dilutions

Microorganism	Drug	Drug Dilution showing MIC value	Concentration of Drug showing MIC value
Bacteroides fragilis	Povidone iodine 5%	1:10	5 mg/ml
	Povidone iodine 10%	1:100	1 mg/ml
	Povidone iodine 5% + Metronidazole 1%	1:100	500 µg/ml + 100 µg/ml
	Povidone iodine 5% + Ornidazole 1%	1:1500	33.33 µg/ml + 6.67 µg/ml
Clostridium perfringens	Povidone iodine 5%	1:50	1 mg/ml
	Povidone iodine 10%	1:50	2 mg/ml
	Povidone iodine 5% + Metronidazole 1%	1:50	1 mg/ml + 200 µg/ml
	Povidone iodine 5% + Ornidazole 1%	1:100	500 µg/ml + 100 µg/ml
Peptostreptococcus magnus	Povidone iodine 5%	1:200	250 µg/ml
	Povidone iodine 10%	1:200	500 µg/ml
	Povidone iodine 5% + Metronidazole 1%	1:500	100 µg/ml + 20 µg/ml
	Povidone iodine 5% + Ornidazole 1%	1:200	250 µg/ml + 50 µg/ml

Zones of inhibition less than 6 mm were not considered due to the size of the well. In a second set of experiments, additional dilutions of drug concentrations were carried out to precisely confirm the MIC value. MIC values were calculated as concentration of drug giving minimum zone of inhibition but more than 6mm. Values of zone of inhibitions of different drug dilutions are described in Table III. Table IV describes the MIC value of different drug solutions and their corresponding concentrations. For *Bacteroides fragilis*, Povidone iodine 5% gave MIC value in dilution of 1:10 (5 mg/ml). While the same for Povidone iodine 10% was found to be 1:100 (1 mg/ml). Combination of Metronidazole 1% and Ornidazole 1% with Povidone iodine 5% gave MIC in dilution of 1:100 (500 µg/ml + 100 µg/ml) and 1:1500 (33.33 µg/ml + 6.67 µg/ml), respectively, which was significantly reduced as compared to Povidone iodine alone. Similarly, for *Clostridium perfringens* the MIC value was observed in dilution of 1:50 for Povidone iodine 5% (1 mg/ml), Povidone iodine 10% (2 mg/ml) and Povidone iodine 5% together with Metronidazole (1 mg/ml + 200 µg/ml). However, for Povidone iodine 5% and Ornidazole 1% combination, the MIC value was observed in dilution of 1:100 (500 µg/ml + 100 µg/ml). In *Peptostreptococcusmagnus*, the MIC value of Povidone iodine 5% (250 µg/ml), Povidone iodine 10% (50 µg/ml) and Povidone iodine 5% with Ornidazole 1% (100 µg/ml + 20 µg/ml) was determined in dilution of 1:200. While the same for Povidone iodine 5% with Metronidazole was found to be 1:500 (250 µg/ml + 50 µg/ml).

DISCUSSION

The effects of anaerobic microbes in the human infections are well known since more than four decades. The management of anaerobic infection is often difficult and can delay their identification by the frequent polymicrobial nature of these infections and by the increasing resistance of anaerobic bacteria to antimicrobials. (Jousimies-Somer *et al*., 2002) Therefore, management of majority of infected wounds which are polymicrobial in nature has gained vital importance. The infections caused by anaerobic bacteria are some of the most important causes of morbidity and mortality in developing countries. (Akhi *et al*., 2013) Also, post-operative infections, which involve both aerobes and anaerobes, may cause severe problems ranging from organ failure to death. Among the antimicrobial agents available for the treatment, as well as prophylaxis against anaerobic infections, the nitroimidazoles such as metronidazole, ornidazole and tinidazole have gained considerable application as potent, nontoxic substances with a narrow antibacterial spectrum. However, for wound infections caused by aerobic bacteria, Povidone iodineis commonly used as an antiseptic treatment. It is widely suitable for surgical asepsis and wound dressing due to its highly soluble nature, low toxicity, and widest bactericidal range. (Zamora, 1986; Lacey and Catto, 1993; Prince *et al*., 1978) Hence, in the present study we have selected some of the most commonly found anaerobic bacteria causing various infections in human beings, which include *Bacteroides fragilis, Clostridium perfringens* and *Peptostreptococcus magnus.* And drugs selected were Povidone iodine 5%, Povidone iodine 10%, Metronidazole 1% and Ornidazole 1%. There are no recent in-vivo or in-vitro studies performed in India or abroad where efficacy of these drug combinations in selected anaerobic isolates has been assessed. To our knowledge this is a novel and pilot study which evaluates efficacy of Povidone iodine in combination with Metronidazole or Ornidazole in anaerobic isolates of *Bacteroides fragilis, Clostridium perfringens, and Peptostreptococcus magnus.* The salient finding of the present study was that the combination of Povidone iodine 5% and Ornidazole 1% was the most effective drug combination in inhibiting the growth of *Bacteroides fragilis and Clostridium perfringens whereas* the combination of Povidone iodine 5% and Metronidazole 1% was more effective in inhibiting the growth of *Peptostreptococcus magnus* than Povidone iodine alone / other drug combinations.

Various in-vitro and in-vivo studies in the past decades have evaluated bactericidal efficacy of Povidone iodine at different concentrations.[18]But the ideal concentration of Povidone iodine for maximal efficacy is not clarified. Some studies have shown that 5% Povidone iodine effectively decreases the bacterial flora of the infected wounds, while other studies have proved more diluted concentration of Povidone iodine was effective. (Dereklis *et al*., 1994; Berkelman *et al*., 1982) However, Nitroimidazoles, including Ornidazole and Metronidazole, are low molecular weight antimicrobial compounds with excellent activity against anaerobic microorganisms. These compounds are usually bactericidal at low concentrations and their spectrum of activity encompasses almost all the anaerobic bacteria. Literature survey suggests that Metronidazole is effective for the management of diverse area of anaerobic infections, such as intra-abdominal infections, gynecologic infections, septicemia, endocarditis, bone and joint infections, central nervous system infections, respiratory tract infections, skin infections, etc. On the other hand, the spectrum of antimicrobial and antiprotozoal activity of Ornidazole is similar to that of Metronidazole, and both agents have similar in vitro activity. (Giamarellou *et al*., 1981) Ornidazole has demonstrated in vitro and in vivo activity against *Bacteroides fragilis* and other Bacteroides spp, Clostridium spp, Peptostreptococcus spp, Peptococcus spp, and Fusobacterium spp. In vivo activity against aerobic organisms (*Enterobacteriaceae, Pseudomonas aeruginosa*, *enterococci*) has also been reported in the presence of anaerobes (mixed aerobic-anaerobic infections). Also a recent clinical evaluation report from India by Vyas *et al*. suggests that the combination of Ornidazole and Povidone – iodine is better as compared to each individual drug in post-surgical wound management. (Vyas *et al*., 2013)

In particular, our study revealed that Povidone iodine 5% and 10% were effective in inhibiting the growth of *B. fragilis* in MIC of 5mg/ml and 1mg/ml respectively. But, addition of Ornidazole 1% (6.67µg/ml) to Povidone iodine 5% significantly reduces the MIC value of Povidone iodine 5% from 5mg/ml to 33µg/ml. Similarly, Povidone iodine 5% in combination with Metronidazole 1% was also shown to be effective in inhibiting growth of B. *fragilis* in much higher MIC value of 500µg/ml & 100µg/ml respectively. This reveals that combination of Povidone iodine 5% and Ornidazole 1% was the most effective drug combination on *B. fragilis.* Similarly, the same drug combination was also most effective on *C. perfringens*, giving MIC value in concentration of 500µg/ml and 100µg/ml. On the same bacteria, Povidone iodine 5% alone gave MIC value of 1 mg/ml, Povidone iodine 10% gave MIC value of 2mg/ml and Povidone iodine 5% in combination with Metronidazole 1% gave MIC value of 1 mg/ml and 200µg/ml. Thus the addition of Ornidazole 1% to Povidone iodine 5% reduces the MIC value of Povidone iodine to half as compared to when it is used alone. However, on *Peptostreptococcus magnus*, the combination of Povidone iodine 5% and Metronidazole was more effective than other drug/ drug combinations, as it gave MIC value in concentration of 100µg/ml and 20µg/ml, respectively. While the MIC value of Povidone iodine 5% alone, Povidone iodine 10% alone and Povidone iodine 5% in combination with Ornidazole 1% were 250 µg/ml, 500 µg/ml and 250 µg/ml + 50 µg/ml, respectively. This shows that addition of Metronidazole 1% to Povidone iodine 5% reduces the MIC value to 2.5 times lower as compared to when it is used alone. Thus in *Peptostreptococcus magnus* infections, this combination is more beneficial as compared to other drugs used in the present study. There are few shortcomings in this study. First is the efficacy of drug combination was studied only in selected organisms. Due to involvement of numerous other microorganisms in wound infections, it is important to carry out similar studies in the other microorganisms too. Secondly, as in-vitro studies are not enough to prove applicability of these drug combinations in management of wound infections, the in-vivo animal studies and clinical trials are also necessary to corroborate their utility in treatment and prevention of wound infection. Finally, the agar dilution method used here was time-consuming and a considerable expertise were required for the accuracy of the results. Thus the same drug combinations and anaerobes should be tested with new and modern methods for the confirmation of the results obtained from our study. We conclude that addition of Ornidazole or Metronidazole to Povidone iodine was more effective, than Povidone iodine alone, in inhibiting the growth of all tested anaerobic bacteria namely *Bacteroides fragilis, Clostridium perfringens, and Peptostreptococcus magnus*. Also, when used in combination, the concentration of Povidone iodine was substantially low. This finding is significant as the dose of Povidone iodine can be reduced to prevent its side effects. These findings support the idea of using combination of different drugs is better as compared to each individual drug in prevention of wound infection and promoting wound healing. Thus, results of our investigation may have important applications to clinical medicine. Further detailed in-vitro and in-vivo clinical studies are necessary to practice these combinations of drugs for the routine therapeutic treatments of various wound infections.

Acknowledgement

We would like to acknowledge the collaboration and commitment of Ethicare Clinical Trial Services and investigators and their staff.

REFERENCES

Akhi, M.T., Shirinzadeh M., Ghotaslou R., Sorous, M.H., Pirzadeh T., Behzad M.N. 2013. Determination of antibiotic sensitivity of Bacteroidfragilis isolated from patients and healthy individuals in Imam Reza Center of Medical Teaching and Treatment-Tabriz. *Jundishapur J. Microbiol*., 6: e7880-e7880. DOI:10.5812/jjm.7880

Berkelman RL, Holland BW, Anderson RL.1982. Increased bactericidal activity of dilute preparations of povidone-iodine solutions. *J ClinMicrobiol*.,15:635–9

Bowler P. G., Duerden B. I. and Armstrong, D. G. 2001. Wound microbiology and associated approaches to wound management. *Clinical Microbiology Reviews*, p. 244–269

Bowler, P. G. 1998.The anaerobic and aerobic microbiology of wounds: a review. Wounds. 10:170–178.

Bowler, P. G., and Davies B. J.1999. The microbiology of acute and chronic wounds. Wounds. 11:72–79.

Brook I. Anaerobic infections: diagnosis and management. Informa Healthcare USA, Inc, New York, NY. 2007.

Daltrey, D. C., Rhodes B. and Chattwood J. G. 1981. Investigation into the microbial flora of healing and non-healing decubitus ulcers. *J. Clin. Pathol*., 34:701–705.

Dereklis DL, Bufidis TA, Tsiakiri EP, Palassopoulos SI.1994. Preoperative ocular disinfection by the use of povidone-iodine 5%. ActaOphthalmol (Copenh).,Oct;72(5):627-30.

Finegold SM. Anaerobic bacteria in human disease. Academic Press, New York, NY. 1977.

Giamarellou H, Gelhoff-Volanaki M, Avlami A, Kanellakopoulou K. and Daikos GK.1981.Ornidazole as a single drug in the treatment of mixed aerobic-anaerobic infections. *J Antimicrob Chemother*., 7(5):569-74.

Gilliland, E. L., N. Nathwani, C. J. Dore, and J. D. Lewis.1988. Bacterial colonisation of leg ulcers and its effect on the success rate of skin grafting. *Ann. R. Coll. Surg. Engl*.,70:105–108.

Hentges DJ.1993. The anaerobic microflora of the human body. Clin. Infect. Dis., 16(Suppl 4):S175–S180.

Jousimies-Somer HR, Summanen P, Baron EJ, Citron DM, Wexler HM, Finegold SM. Wadsworth-KTL anaerobic bacteriology manual, 6th ed. Star Publishing, Belmont, CA. 2002.

Lacey RW, Catto A.1993. Action of povidone-iodine against methicillin-sensitive and-resistant cultures of Staphylococcus aureus. *Postgrad Med J*., 69:S78–83.

Nagy E.2010. Anaerobic infections: update on treatment considerations. *Drugs*., 70:841–858.

Nichols, R. L., and J. W. Smith.1994. Anaerobes from a surgical perspective. *Clin. Infect. Dis*., 18:S280–286.

Prince HN, Nonemaker WS, Norgard RC, Prince DL.1978. Drug resistance studies with topical antiseptics. *J Pharm Sci*., Nov;67(11):1629-31.

Scott E.M., Garman S.P. and Mcgrath S.J. 1982. An assessment of the fungal activity of antimicrobial agents used for hard surface and skin disinfection" *J.ClinHosp.Pharma*., 11(3):199-205.

Twum-Danso K, Grant C, al-Suleiman SA, Abdel-Khader S, al-Awami MS, al-Breiki H, Taha S, *et al*.1992. Microbiology of postoperative wound infection: a prospective study of 1770 wounds. *J Hosp Infect*., 21(1):29-37.

Vyas D, Shah M, Walwaikar P. P. and Mahajan B.2013. Clinical Trial Comparing Efficacy And Safety Of Ornidazole 1% And Povidone iodine 5% Fixed Dose Combination Ointment with Povidone iodine 5% formulation and Ornidazole 1% Formulation for Prevention or Treatment of Surgical Wounds. *Indian Medical Gazette*., 265-267.

Werner H, Kuchenbecker K, Hammann R. 1983. Efficacy of tinidazole against anaerobes in comparison with metronidazole, ornidazole, cefoxitin and lamoxactam. ImmunInfekt., 11(4):143-7. German. PubMed PMID: 6681175

Zamora JL.1986. Chemical and microbiologic characteristics and toxicity of povidone-iodine solutions. *Am J Surg*., 151:400–6.

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