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RESEARCH ARTICLE

COMPARATIVE IN VITRO ACTIVITY OF MEBATIC AGAINST CLINICAL ISOLATES

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ABSTRACT

Study was aimed to explore the *in vitro* effect of ofloxacin and ornidazole (Mebatic) combination as compared with other drugs on aerobic and anaerobic bacteria. The study was done from March 2014 to March 2015 in the Department of Microbiology, Venus Medicine Research Centre, Baddi, Himachal Pradesh. A total of 1059 clinical samples were collected in a sterile container for aerobic and anaerobic culture. Identification of bacteria was done by microscopy and biochemical reaction. The antibiotic sensitivity testing was performed by cup plate method.

Out of 1059 samples, 605 samples showed the growth of bacteria. Further identification revealed the presence of nine different Gram negative and Gram positive organisms such as included *S. aureus, S. pneumoniae*, *C. trachomatis*, *N. gonorrhoeae Proteus spp.*, *C. perfringens*, *Bacteroides spp.*, *Yersinia spp.* and *Fusobacterium spp*. The susceptibility of ofloxacin plus ornidazole (Mebatic) against *S. aureus*, *S. pneumoniae*, *N. gonorrhoea*, *C. trachomatis*, *Proteus spp.*, *Fusobacterium* spp, *C. perfringens*, *Bacteroides spp.* and *Yersinia spp.* was > 85% which was high compared to metronidazole, clindamycin alone and, ceftriaxone plus metronidazole, levofloxacin plus metronidazole, moxifloxacin plus metronidazole in combinations. A high resistance to metronidazole (30-100%), clindamycin (25-92.6%), ceftriaxone plus metronidazole (39-88.4%), levofloxacin plus metronidazole (23-83.0%), ofloxacin plus metronidazole (16-67%) and moxifloxacin/metronidazole (18.9%-75.2%) was observed.

The study concluded that all the pathogens were found to be highly susceptible to ofloxacin/ornidazole compared to other antibiotics.

INTRODUCTION

The fluoroquinolone antibiotics are among the most widely used human medicine with a broad spectrum activity against most Gram-negative and Gram-positive bacteria. Quinolones rapidly inhibit deoxyribonucleic acid (DNA) synthesis by promoting cleavage of bacterial DNA in the DNA-enzyme complexes of DNA gyrase and type IV topoisomerase, resulting in rapid bacterial death (Hooper, 2000). Imidazole such as ornidazole and metronidazole is known to be quite active against Gram-negative anaerobes such as *Bacteroides fragilis, Prevotella melaninogenica, Prevotella disiens, Prevotella oralis, Prevotella intermedia* and *Fusobacterium spp.* but does not appear to possess any clinically relevant activity against facultative anaerobes or obligate aerobes. The anaerobic gram-positive sporing bacilli such as *Clostridium spp.* are nearly always susceptible to imidazole (Neu 1991; Dollery 1999). Quinolones is active against aerobic and some anaerobic bacteria (King *et al*., 1982).

Antibiotic resistance among anaerobes has steadily increased since the early 1970s. Initially, antibiotic resistance among anaerobic bacteria went unnoticed for several reasons, predominantly because many mixed infections (David, 2004). The increase in antibiotic resistance among anaerobes has spawned intensive investigation into the mechanisms of resistance and resistance-gene transfer. The most frequently isolated antibiotic-resistant anaerobe is *Bacteroides fragilis*. Resistance is also seen among anaerobes that were previously considered to be highly susceptible to fluoroquinolone antibiotics, raising concerns about appropriate empirical therapy. The fluoroquinolones and nitroimidazoles have currently been using extensive worldwide for clinical applications because of their good bioavailability and pharmokinetic profile.

Ofloxacin is (the broad antibacterial spectrum of quinolones) having very high gram-negative activity, including moderate activity against *Pseudomonas aeruginosa* (Khan *et al*., 2008;

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Messadi *et al*., 2008) where most anaerobic pathogens and several Gram-positive strains are moderately susceptible (Miller and Shah, 1997; Messadi *et al*., 2008). To increase the spectrum and to lesser the chances of resistance it was combined with ornidazole, a nitroimidazole with an antibacterial activity against most of anaerobes (Kumar *et al*., 2007; Kurt *et al*., 2008) and its single-dose is an important alternative for the treatment of many infections caused by anaerobes (Saracoglu *et al*., 1998). Combinations of fluoroquinolones with other antimicrobial agents have been investigated extensively. Clinical studies have demonstrated synergy of fluroquinolone with metronidazole (Neu 1991). The present study was conducted to explore the *in vitro* effect of ofloxacin and ornidazole (Mebatic) combination as compared with other drugs on aerobic and anaerobic bacteria.

MATERIAL AND METHODS

Bacterial isolates

The present study was carried out over a period of two years from March 2012 to March 2014 in the Department of Microbiology, Venus Medicine Research Centre, Baddi, Himachal Pradesh. A total of 1059 clinical samples consisting of blood, urine, vaginal swab, dental abscess, pus and intra-abdominal secretions were collected in a sterile container. On receipt of clinical samples, they were transferred into cooked meat media (Hi-Media, Mumbai, India) and cooked meat media were incubated for 40 to 48 hours at 35 ºC in an ambient atmosphere. After incubation, approximately 0.3 ml of the enrichment broth was aspirated and plated onto pre-reduced CDC anaerobic agar with 5% sheep blood (C plate), CDC anaerobic agar with 5% sheep blood and PEA, and CDC anaerobic agar with laked sheep blood, kanamycin and vancomcyin (all media from BD, USA) for anaerobic culture. Then, all plates were incubated at 35 to 37 ºC in an anaerobic incubator (Thermo Forma, USA). A separate C plate was simultaneously inoculated, and incubated in 5% CO2 for 40 to 48 hours for growth comparison. Culture plates were removed after 40 to 48 hours incubation for inspection of growth, and re-incubated for a total of 6 days. Suspected growth of anaerobes was confirmed by aero-tolerance testing (Silva *et al*., 2003). For aerobic culture, subculture was done on blood agar, MacConkey agar and chocolate agar. Blood agar and Chocolate agar were incubated under 5-10% CO2 . The growth of organisms was identified by colonial morphology, gram staining and biochemical tests as described earlier (Cheesbrough, 2000).

Antimicrobial susceptibility testing

Antibiotic susceptibility testing for metronidazole, clindamycin, ceftriaxone plus metronidazole, levofloxacin plus metronidazole, moxifloxacin plus metronidazole, and ofloxacin plus ornidazole (Mebatic) were determined with cup plate method as described by Bennet *et al*., (1966). Testing was performed using Brucella agar plates supplemented with 5 g of hemin and 1 g of vitamin K per millilitre plus 5% laked sheep blood. The plates were incubated for 48 h at 37 ºC in anaerobic jars. The *Bacteroides fragilis* ATCC 25285 was used as a control.

RESULTS AND DISCUSSION

A total of 1059 clinical samples of urine, blood, vaginal swab, dental abscess, intraabdominal secretion and pus were collected from various hospitals across India and were processed for isolation of pathogenic bacteria. Out of the samples analyzed, 605 samples showed the growth (Table 1). Among the samples that showed the presence of pathogens, around 47.5 % samples were of urine followed by blood (22.5 %), vaginal swab (18.2%), dental abscess (5.8%), intra abdominal secretion (5.5%) and pus (1.3%) (Table 1).

Table 1A profile of clinical samples used as a source of the pathogenic isolates

Clinical Samples	Total clinical samples	Samples showing growth	Prevalence (%)
Urine	389	284	47.5
Blood	289	136	22.7
Vaginal Swab	176	109	18.2
Dental abscess	134	35	5.8
Inta-abdominal secretion	49	33	5.5
Pus	22	8	1.3

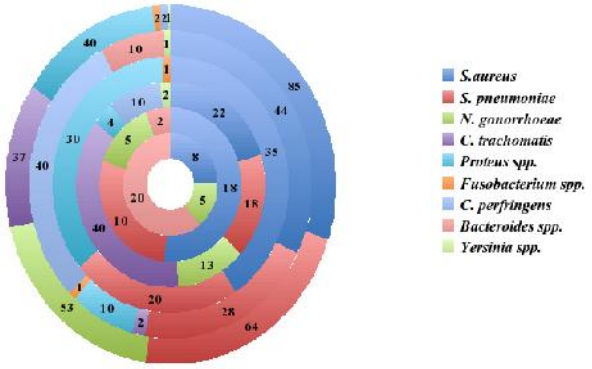

Figure 1 Profile of different clinical isolates isolated from various samples.

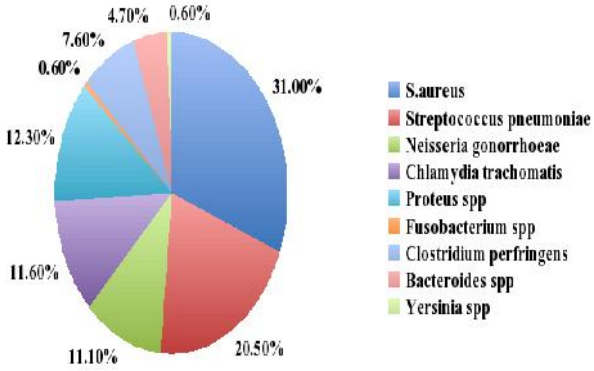

Figure 2 Prevalence of various pathogens.

Morphological and biochemical characterization of the samples revealed the presence of nine different Gram negative and Gram positive organisms that included *S. aureus, S. pneumoniae, C. trachomatis, N. gonorrhoeae, Proteus spp., C. perfringens, Bacteroides spp., Yersinia spp.* and *Fusobacterium spp*. The detailed profile of various organisms collected from various clinical samples is shown in Figure 1. Among the isolates, *S. aureus* (29.9%) was found to be the most dominant pathogen Figure 2. Several studies from India demonstrated the prevalence of *S. aureus* to be 34 to 54% in various clinical

samples (Arora *et al*., 2010; Goplakrishnan and Suresh 2010; Mathews *et al*. 2010; Singh *et al*., 2013; Rezvan *et al*., 2009). A study performed by Rao *et al*. (2013) reported the prevalence of *S. pneumoniae* to be 27.8% which is similar to the current study where its prevalence rate was 20.1%. Jensen *et al*. (2003) reported 11.5% prevalence of *C. trachomatis* in his study, which is almost similar to our study (13%). Oknkow *et al*. (2014) showed that out of the total number of clinical samples, *N. gonorrhoeae* was detected in 15.2% of samples followed by *Bacteriods spp.* was (5.2%), and *Yersinia spp*, (0.67%) which are comparable to current investigation.

The enhanced activity of Mebatic may be due to synergistic activity of ofloxacin and ornidazole. According to Bhardwaj *et al*. (2003) combination of a quinolone drug with a nitroimidazole drug enhances the antimicrobial properties against both aerobic and anaerobic bacterial infections.

A high prevalence of resistance to metronidazole (30-100%), clindamycin (25-92.6%), ceftriaxone plus metronidazole (39-88.4%), levofloxacin plus metronidazole (23%-83.0%) and moxifloxacin/metronidazole (18.9%-75.2%) was observed.

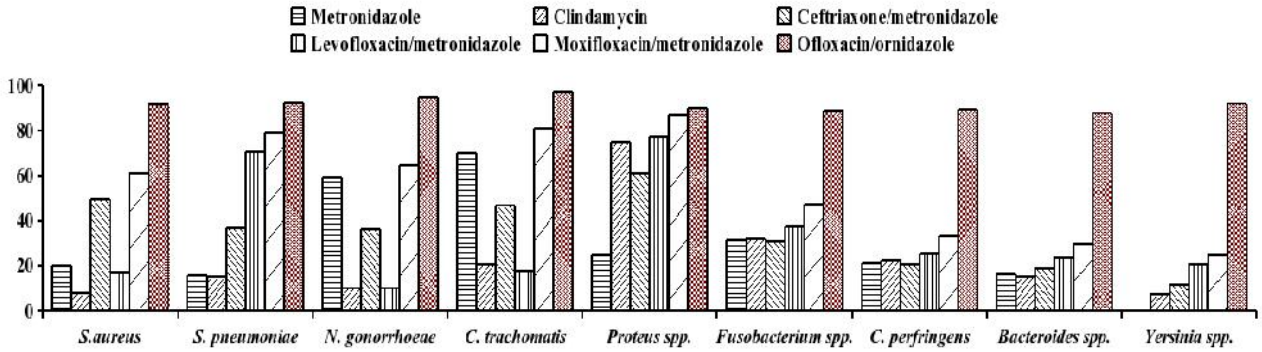

Figure 3 Susceptibility pattern of Gram-negative and Gram-positive pathogens isolated.

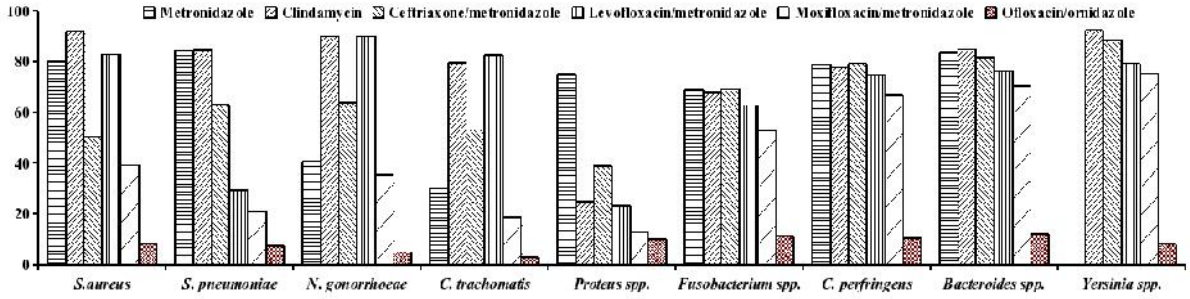

Figure 4 Resistance pattern of Gram-negative and Gram-positive pathogens isolated.

Feglo *et al*. (2010) noted that *C. perfringens* was most prevalent pathogen (76.9%) in blood followed by *Fusobacterium spp.* (33.3%), *Bacteroides spp.* (31.2%), and *Yersinia* spp. (25%). Foulon *et al*. (2003) also showed that prevalence of *Bacteroides spp.* in blood samples was 26.2%, which is in accordance with the results of the present study. In vaginal swab samples prevalence of *C. trachomatis* was highest, accounting for 50.6%, whereas *Bacteroids spp.* contribute 62.5% in intra-abdominal secretions. In pus samples all the organisms contributed less significantly.

Antibiogram profile for all the pathogens isolated from various clinical samples is presented in Figure 3 and 4. In our study we found that the activity of ofloxacin plus ornidazole (Mebatic) was the highest among all the tested antibiotics. The susceptibility of ofloxacin plus ornidazole (Mebatic) against *S. aureus, S. pneumoniae, N. gonorrhoea, C. trachomatis, Proteus spp., Fusobacterium* spp, *C. perfringens, Bacteroides spp.* and *Yersinia spp.* was 91.7%, 92.6%, 95%, 97% 86.7%, 88.9%, 88.4%, 87.8%, and 92%, respectively which was high compared to metronidazole, clindamycin alone and, ceftriaxone plus metronidazole, levofloxacin plus metronidazole and moxifloxacin plus metronidazole in combinations.

Dubruil *et al*. (1988) conducted a study on the 58 anaerobic strains (*Bacteroides spp*.) and observed that the combination of metronidazole plus ofloxacin had an additive bacteriostatic effect on 30 strains and a synergistic effect on 26 strains. No antagonism was noted with any strain.

In the current investigation, *S. aureus* showed 92% resistance to clindamycin alone which is very high compared to previous study of Reddy *et al*. (2014) where they noticed that nearly 42.6% of *S. aureus* isolates were resistance to clindamycin. Recent data from Yardeni *et al*. (2013) reported that single daily dosing of ceftriaxone plus metronidazole is as safe and effective as the triple antibiotic regimen (ampicillin, gentamicin and metronidazole) and has significant advantages for the conservative therapy of complicated appendicitis in children. Similar experiments for levofloxacin plus metronidazole combination have been performed by Kelly *et al*. (2004) where they showed that levofloxacin (750 mg) and metronidazole (1,500 mg) may offer a more convenient once-daily treatment option for mixed aerobic-anaerobic infections, compared to the standard administration of metronidazole (500 mg every 8 h), however, the result of the current findings state that these combinations are developing high resistance which may be due to overuse.

Among all antibiotics tested, Mebatic revealed the highest activity against aerobic and anaerobic, Gram-negative and Gram-positive organisms. Further studies are needed to evaluate these combinations in clinical settings as they can play important role for the clinicians as viable alternative to fluoroquinolones in scenario of rising resistance to fluroquinolones moieties.

CONCLUSION

As a result of decline in research on antibiotics and the problem of antibiotic resistance growing steadily, there is a need for newer and novel combinations of antibacterial agents. As the administration of ofloxacin and ornidazole has been reported to have favorable pharmacokinetic properties, the combination of ofloxacin and ornidazole has therefore, all the advantages needed for an effective antimicrobial therapy. Thus results of the present study concluded that the fixed dose combination of ofloxacin and ornidazole is an alternative and effective option for use in many mixed microbial infections occured due to aerobic and anaerobic bacteria and pathogenic protozoa when compared to other antibiotic combinations, which might be due to resistance countering potential of Mebatic.

References

Arora S, Devi P, Arora U, Devi B, 2010, Prevalence of Methicillin- resistant *Staphylococcus aureus* (MRSA) in a tertiary care hospital in northern India. *J. Lab. Physicians.*, **2:**78-81.

Bennet JR, Brodie JL, Benner EJ, and Kirby WMM (1966) Simplified, accurate method for antibiotic assay of clinical specimens. Appl Microbiol 14:170-177.

Bharadwaj R, Vidya R, Dewan B, Pal A, 2003. An *in vitro* study to evaluate the synergistic activity of norfloxacin and metronidazole. *Indian J. Pharmacol.,* **35:** 220-226.

Cheesbrough M (2000). Biochemical tests to identify bacteria. In: District laboratory practice in tropical countries. 2nd ed. Cambridge University Press, UK,178-187.

Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twenty-third informational supplement. CLSI document M100- S23. 2013;Wayne, PA 19087 USA.

David WH, 2004, Prevalence of Antibiotic Resistance in Anaerobic Bacteria: Worrisome Developments. *Clin. Infect. Dis.*, **39:** 92-97.

Dharanidharan R, Pazhani GP, Sarkar A, *et al*., 2013, Case-control study on the role of enterotoxigenic *Bacteroides fragilis* as a cause of diarrhea among Children in Kolkata, India. *PLoS One*. **8:** e60622.

Dollery C, 1999, Therapeutic Drugs. 2nd ed. Churchill Livingstone: Edinburgh; N137-40, M146-50.

Dubreuil L, Devos J, Beerens H, Romond C, 1988, In vitro activity of an ofloxacin- metronidazole combination against anaerobic bacteria. Kinetics of the action of metronidazole against *Bacteroides fragilis. Pathol. Biol*., **36:**488-92.

Feglo PK, Stephen YG, Quay SNA, Adu-Sarkodie Y, Opoku-Okrah C, 2010, Occurrence, species distribution and antibiotic resistance of *Proteus* isolates: a case study at the Komfo Anokye Teaching Hospital (KATH) in Ghana. *Int. J. Pharma Sci. Res.*, **1:** 347- 352.

Foulon I, Pierard D, Muyldermans G, Vandoorslaer K, Soetens O, Rosseel P, Lauwers S, 2003, Prevalence of fragilysin gene in *Bacteroides fragilis* isolates from blood and other extraintestinal samples. *J. Clin. Microbiol*., **41:** 4428–4430.

Gopalakrishnan R, Sureshkumar D, 2010, Changing trends in antimicrobial susceptibility and hospital acquired infections over an 8 year period in a tertiary care hospital in relation to introduction of an infection control programme. *J. Assoc. Physicians. India*, 58 (Suppl): 25-31.

Hooper D. Quinolones. In: Mandell GL, Bennett JE, Dolin R, 2000, Mandell, Douglas, and Bennett's principles and practice of infectious diseases. 5th ed. Philadelphia: Churchill Livingstone, 404–23.

Jensen IP, Foghand H, Prag J, 2003, Diagnosis of Chlamydia trachomatis infections in a sexually transmitted disease clinic: evaluation of a urine sample tested by enzyme immunoassay and polymerase chain reaction in comparison with a cervical and/or a urethral swab tested by culture and polymerase chain reaction. *Clin. Microbiol. Infect.,* **9:**194–201.

Khan JA, Iqbal Z, Rahman SU, Farzana K, Khan A, 2008, Report: Prevalence and resistance pattern of Pseudomonas aeruginosa against various antibiotics. *Pak. J. Pharm. ci.,* **21:**311-315.

King A, Warren C, Shannon K, 1982, *In vitro* antibacterial activity of norfloxacin (MK-0366). *Antimicrob. Agents Chemother*., **21:**604-7.

Kumar YS, Ramesh S, Rao YM, Paradkar AR, 2007, Effect of rifampicin pretreatment on the transport across rat intestine and oral pharmacokinetics of ornidazole in healthy human volunteers. Drug Metabol. Drug Interact., **22:**151-163.

Kurt O, Girginkardesler N, Balcioglu IC, Ozbilgin A, Ok UZ, 2008, A comparison of metronidazole and single-dose ornidazole for the treatment of dientamoebiasis. *Clin. Microbiol. Infect.,* **14:** 601-604.

Mathews AA, Thomas M, Appalaraju B, Jaylakshmi J, 2010, Evaluation and comparison of test to detect methicillin resistant *S.aureus*. *Indian J.Pathol. Microbiol.,* **53:**79-82

Messadi AA, Lamia T, Kamel B, Salima O, Monia M, Saida BR, 2008. Association between antibiotic use and changes in susceptibility patterns of *Pseudomonas aeruginosa* in an intensive care burn unit: A 5-year study, 2000-2004. Burns, **34:**1098-1102.

Miller JMH, Shah S, 1997. Activities of ciprofloxacin, levofloxacin, ofloxacin and sparfloxacin against speciated coagulase-negative staphylococci sensitive and resistant to fluoroquinolones. *Int. J. Antimicrob. Agents*, **9:** 127-130.

Neu HC, 1991, Synergy and antagonism of combinations with quinolones. *Eur. J. Clin. Microbiol. Infect. Dis.*, **10**:255- 61.

Okonkwo EC, Ogbu O, Anyim C, Nworie O, Orji JO, Nwuzo AC, 2014, Microbiology prevalence of *Neisseria Gonorrhoea* infection by PCR method using urine samples from HIV sero-positive women. *Int. J. Sci. Res.*, **3:**364-366.

Rao R, Basu R, Sarkar A, Bidyarani K, 2013, Prevalence and antimicrobial susceptibility pattern of *Streptococcus Pneumoniae* isolated from respiratory samples in a South Indian tertiary care hospital. *Int. J. Health Sci. Res.*, **3:** 121-126.

Reddy M, Hima C, Bindu M, Soumendranath M, Kanta RC, Kapur I, 2014, Prevalence of inducible clindamycin resistance in *Staphylococcus aureus* from clinical samples: a study from a teaching hospital in Andhra Pradesh, India. *Int. J. Curr. Microbiol. App. Sci.,* **3:**402-409.

Rezvan M, Musav GA, Fadavi N, 2009, The prevalence of nasal carriage methicillin- resistant *Staphylococcus aureus* in hospitalized patient. *Pak. J. Med. Sci*., **25:** 656- 659.

Saracoglu F, Gol K, Sahin I, Turkkani B, Atalay C, Oztopcu C, 1998, treatment of bacterial vaginosis with oral or vaginal ornidazole, secnidazole and metronidazole. *Int. J. Gynaecol. Obstet.*, **62:** 59-61.

Silva VL, Carvalho MA, Nocoli JR, Farias LM, 2003, Aerotolerance of human clinical isolates of *Prevotella spp. J. Appl. Microbiol.*, **94:** 701-707.

Singh MP, Sharma SK, Shukla S, Pandit NP, 2013, Prevalence rate and antibiotic susceptibility test (AST) pattern of Methicillin resistant *Staphylococcus aureus* (MRSA) isolates from different clinical specimens of Teerthankar Mahaveer Hospital, Moradabad, India. *Int. J. Curr. Microbiol. Appl. Sci.*, **2:** 307-314.

Yardeni D, Kawar B, Siplovich L, Rosine I, Zebidat M, *et al*., 2013, single daily dosing of ceftriaxone and metronidazole is as safe and effective as ampicillin, gentamicin and metronidazole for non-operative management of complicated appendicitis in children. *Pediat. Therap.*, **3:**177.

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