REPORT

ANTIBACTERIAL EFFECT OF NSAIDS ON CLINICAL ISOLATES OF URINARY TRACT INFECTION AND DIABETIC FOOT INFECTION

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ASTRACT

Non Steroidal Anti-inflammatory Drugs (NSAIDS) are a group of chemically dissimilar agents that have primary effect of inhibition of prostaglandin's synthesis. Aspirin (Acetyl Salicylic Acid) is used as an analgesic, anti pyretic, anti-inflammatory agent and also have an anticoagulant effect. Tylenol (acetaminophen) is used as pain reliever. The objective of this study was to determine the effect of Aspirin and Tylenol against clinical isolates of urinary tract infection (UTI) and diabetic foot infections.

A total of 100 clinical isolates were analyzed. Out of these 50 were urine samples from diabetic patients with UTI and 50 pus samples from diabetic foot infection. Bacteriological study was done by inoculating urine samples on Cysteine Lactose Electrolyte deficient (CLED) media. Pus samples were inoculated on Blood agar and MacConkey's agar. Identification was done by colony characteristics, gram staining and standard biochemical tests and Quick Test Strip (QTS-20)DESTO Laboratories, Karachi. Antibacterial effect of Aspirin and Tylenol were tested against 100 clinical isolates by Replica plate method, Agar well diffusion method and tube dilution method. Concentrations of Aspirin and Tylenol (10 µg, 50 µg, 100 µg, 500 µg, 1000 µg) were made in Muller Hinton media.

Bacteria isolated from urine samples were *Escherichia coli* 30%, *Staphyloccus aureus* 20%, *Entrococcus faecalis* 10%, *S.saprophyticus*10%, Proteus spp 6%, Pseudomonas spp.6%, *S. pyogenes* 6%, *S. agalactiae* 6%, *S.epidermidis* 4%, and *Klebsiella* spp. 2%. Bacteria isolated from pus samples were S *aureus* 30%, *Pseudomonas aeruginosa* 18%, *S.epidermidis* 14%, *Klebsiella pneumonia*12%, *Proteus mirabilis* 12%, *E. coli* 10%, *P.vulgaris* 4%. Aspirin was effective at 100-500 µg concentration against all isolates. Tylenol has marked effect on pathogens at 100 µg concentration. Aspirin and Tylenol along with analgesic, anti-pyretic, anti-inflammatory properties also have marked anti bacterial effect on isolates from UTI and Diabetic foot infections and inhibits the growth of both gram negative and gram positive bacteria, and both can be used synergistically with antibiotics for effective treatment.

Keywords: NSAIDS, aspirin, tylenol, diabetic foot infection, UTI.

INTRODUCTION

NSAIDs are non narcotic drugs with analgesic, antipyretic and anti-inflammatory effects which relieve pain, fever and inflammation. The term "non-steroidal" is used to distinguish these drugs from steroids which have a similar eicosanoid-depressing, anti-inflammatory action (Dennis, 1995).

Most NSAIDs act as non-selective inhibitors of the enzyme cyclooxygenase, inhibiting both the cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) isoenzymes (Julia *et al.,* 2005). Cyclooxygenase catalyses the formation of prostaglandins and thromboxane from arachidonic acid derived from the cellular phospholipid bilayer by phospholipase A_2. Prostaglandins which act as messenger molecules in the process of inflammation. (Vane, 1971).NSAIDs is usually indicated for the treatment of acute or chronic conditions where pain and inflammation are present. Research continues into their potential for prevention of colorectal cancer and cardiovascular diseases (Monica, 2007) Aspirin and Tylenol, are over-the-counter analgesic used worldwide for more than four decades. Acetaminophen is regarded as safe and effective drug. . Like other r NSAIDs it interfere with growth of both Gram negative and Gram positive bacteria in vitro and has inhibitory effect on *E. coli*, *S. saprophyticus* (Cederlund and Per-Anders, 1993), *Pseudomonas cepacia*,(Burns*et al.*, 1989) and *S. aureus* (Christopher *et al.,* 1999) In some organisms, increased resistance or susceptibility to several antibiotics can be induced by growing in a sub inhibitory (below inhibitory level of drug) concentration of Aspirin (Aumercier *et al.,* 1990; Graham *et al.*,1989). *H. pylori* was not susceptible to aspirin and other NSAIDs, like indomethacin, ibuprofen, naproxen in vivo (Kopp and Ghosh, 1994). Sodium salicylate inhibits biofilm formation by *P. aeruginosa* and *S. epidermidis* on contact lenses and medical polymers such as polyethylene and polystyrene. Bacterial adhesion also decreases in a dose-dependent manner (Muller *et al.,* 1998). Coating of catheters with Salicylate or incorporation of salicylate

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into contact lens solutions might decrease the incidence of some device-related infections (Muller *et al.*, 1998; Robert, and Aristidis, 1996).

Treatment of an invasive experimental *S. aureus* infection in endocarditis with intravenous Aspirin caused a significant reduction in bacterial densities within target tissues and these effects are mediated by salicylic acid (Bosetti *et al.,* 2006). The salicylate analogue *p*-aminosalicylate (PAS) act as an effective anti tuberculosis agent used as a second-line TB drug (Teichberg *et al.*, 1993).

Urinary tract infections (UTI) are the most common of all bacterial infections affecting human beings through out their life span. The bacteria, which are able to attack the urinary tract n in symptomatic urinary tract infections, have a wide range of virulence factors to evade the host defenses. Ninety percent of the uncomplicated UTI are caused by *E. coli*. Not only is UTI common but the range of clinical effects it can produce are significant. However, the human urinary tract is well sheltered against the bacterial invasion by specific and non specific immune defense systems, under normal conditions (Rama *et al.*, 2005).

Spectrum of microbial flora in infected foot of diabetic patient like ulcer is a common cause of morbidity leading to severe complications like gangrene and amputations (Bansal *et al.,* 2008).The objective of this study was to determine the effect of Aspirin and Tylenol (NSAIDS) on the growth of isolates of UTI and diabetic foot infection.

MATERIALS AND METHODS

A total of 100 clinical isolates were analyzed. Out of these 50 samples were of UTI and 50 from Diabetic foot infection. Pus and urine samples were collected from different hospitals of Karachi using standard protocol of sample collection. Bacteriological study was done by inoculating urine samples on Cysteine Lactose Electrolyte deficient (CLED) media (Washington *et al.*, 2005).

Pus samples were inoculated on Blood agar and MacConkey's agar. Identification was done by colony characteristics, gram staining, standard biochemical tests. and Quick Test Strip (QTS-20) DESTO Laboratories, Karachi.

Susceptibility testing

Bacterial cultures were tested against conventional pain killers (NSAIDS) Aspirin and Tylenol by Replica method, Agar well diffusion method and tube dilution method. 30 mg/ml stock solution of Aspirin and 60 mg/ml stock solution of Tylenol was made in sterile distilled water. Working solution of 1000 mg/ml concentration was serially diluted. Concentration of Aspirin and Tylenol (10 µg, 50 µg, 100 µg, 500 µg, 1000 µg) were prepared. Bacterial suspensions of isolated cultures were made in sterile saline and matched with McFarland index 0.5 tubes. Muller Hinton Agar plates were inoculated with different bacterial suspensions. Plates were than bored with 6mm borer.20 µL of all concentrations of Aspirin and Tylenol was loaded into the wells made in the plates. All the plates were incubated for 24 hours at 37^{o}C.

Replica method

Muller Hinton Agar plates were prepared by adding different amount of stock solution required for different concentrations (1000 µg/ml, 500 µg/ml, 100 µg/ml, 50 µg/ml, and 10 µg/ml).

Master plate was prepared by inoculating different cultures on specific location and the plate was incubated for 24 hours at 37^{o}C.

With the help of wooden replica, cultures were transferred. Controls were also inoculated before and after the NSAIDS were added to the plates. All the plates were incubated for 24 hours at 37^{o}C. Next day specific culture location was observed for growth of culture.

Tube dilution method

The tube dilution test is the standard method for determining levels of resistance. Serial dilutions of the NSAIDS were made in Muller Hinton broth which was inoculated with a standardized number of organisms and incubated for a 24 hours. The lowest concentration of drug preventing ce of turbidity is considered to be the minimal inhibitory concentration (MIC). Additionally, the minimal bactericidal concentration (MBC) determined by sub culturing the contents of the tubes on antibiotic-free Muller Hinton Agar and examining for bacterial growth. Although the tube dilution test is correct but the test is laborious because serial dilutions of the antibiotic must be made and only one isolate can be tested in each series of dilutions.

Agar well diffusion methods

Wells in the Muller Hinton Agar plates were made by the help of 6mm borer. The culture was swabbed uniformly across plates and the known concentration of the drug to be tested was added in the well. If the drug is effective against bacteria at a certain concentration, no colonies will grow when the concentration in the agar is greater than or equal to the effective concentration, this is the zone of inhibition. Thus, the size of the zone of inhibition is a measure of the compound's effectiveness: the larger the clear area around the well, the more effective is the compound.

RESULTS

A total of 100 clinical isolates were analyzed. Out of these 50 were urine samples from patients with UTI and

50 pus samples from diabetic foot infection. Fig. 1 Bacteria isolated from urine samples were *E. coli* 30%, *S. aureus* 20%, *E. faecalis* 10%, *S. saprophyticus* 10%, *Proteus spp* 6%, *Pseudomonas spp*. 6%, *S. pyogenes* 6%, *S. agalactiae* 6%, *S.epidermidis* 4%, and *Klebsiella spp*. 2%.

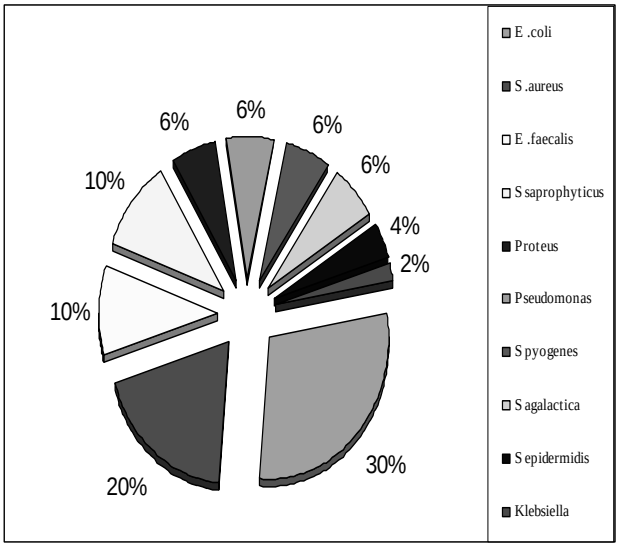

Fig. 1: Isolates of urinary tract infection.

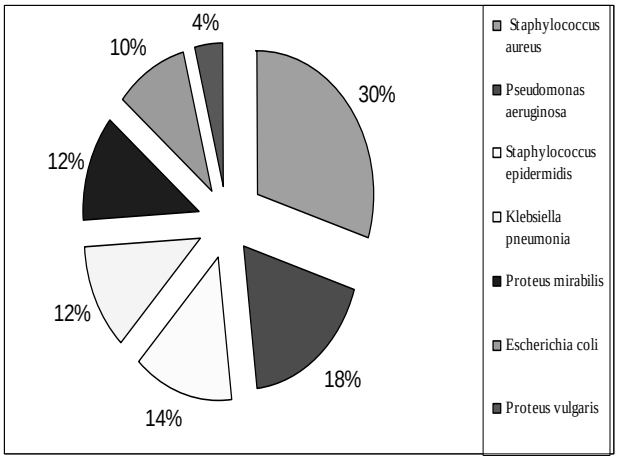

Fig. 2: Isolates of diabetic foot infection.

Antibacterial effect of Aspirin and Tylenol (NSAID) was determined against different bacterial strains isolated from UTI and diabetic foot infection. Figs. 3 and 4 show the effect of Aspirin on isolates of UTI and diabetic foot infection. Aspirin was effective at 500 µg/ml concentrations while Tylenol was effective at 100 µg/ml concentration (figs. 5 and 6).

Statistical analysis was done by the help of Minitab software using one way ANOVA and tables 1and 2 for effect of Tylenol on diabetic foot infection and urinary tract infections respectively while tables 3 and 4 show the effect of Aspirin on diabetic foot infection and UTI isolates respectively demonstrate that results are significant.

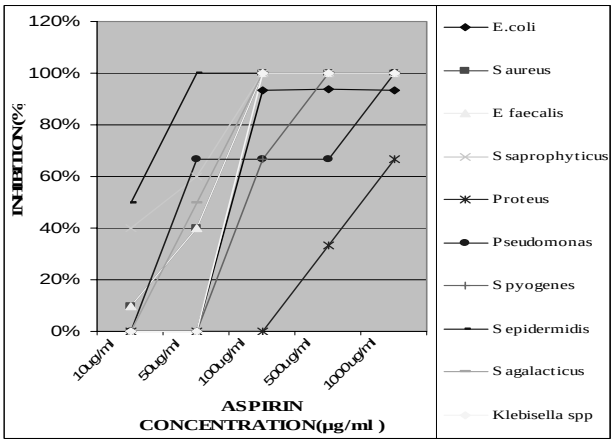

Fig. 3: Effect of Aspirin on isolates of urinary tract infection.

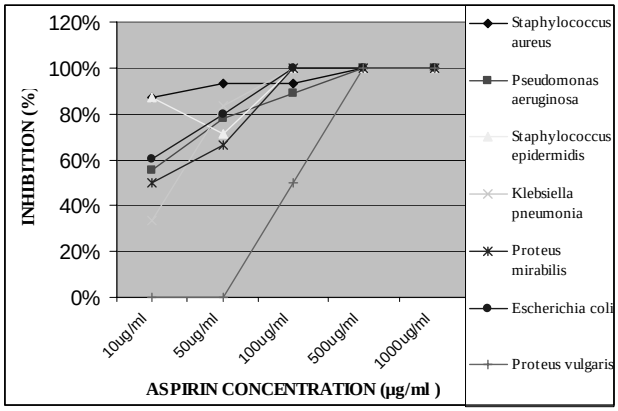

Fig. 4: Effect of aspirin on isolates of diabetic foot infection.

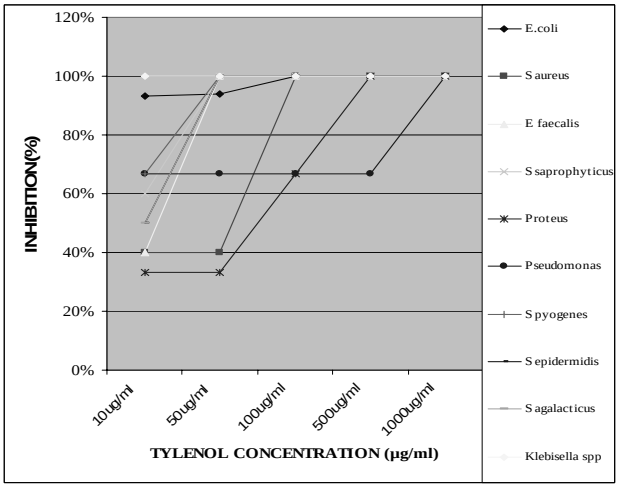

Fig. 5: Effect of Tylenol on isolates of urinary tract infection.

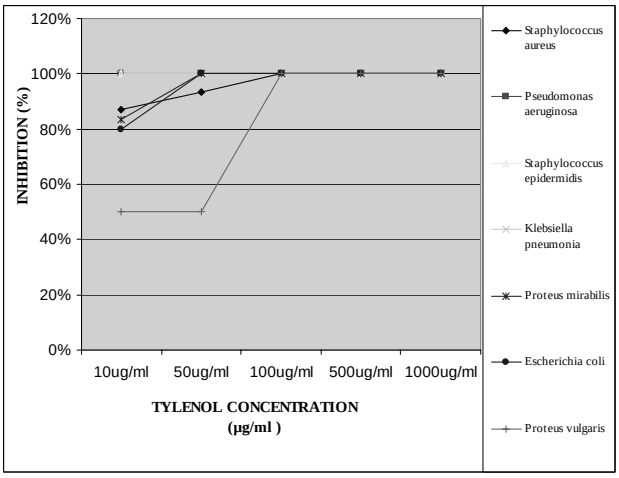

Fig. 6: Effect of Tylenol on isolates of diabetic foot infection.

Table 1: Effect of Tylenol on diabetic foot infection isolates.

Level	Microorganism	Standard Deviation	Mean
1	*S. aureus*	5.87	96.00
2	*Ps. Aeruginosa*	0.00	100
3	*St. epidermidis*	0.00	100
4	*Klebsiella pneumonia*	0.00	100
5	*Proteus mirabilis*	7.60	96.00
6	*Escherichia coli*	8.94	96.00
7	*Proteus vulgaris*	27.39	80.00

One-way analysis of variance
Analysis of variance for C2

Source	DF	SS	MS	F	P
m.o	6	1514	252	1.91	0.113
Error	28	3689	132		
Total	34	5203			

Table 2: Effect of Tylenol on UTI (urinary tract infections) isolates.

Level	Microorganism	Standard Deviation	Mean
1	*E. coli*	3.65	97.33
2	*S. aureus*	32.86	76.00
3	*E faecalis*	26.83	88.00
4	*S. saprophyticus*	17.89	92.00
5	*Proteus*	33.34	66.66
6	*Pseudomonas*	18.26	80.00
7	*S. pyogenes*	14.91	93.33
8	*S. epidermidis*	22.36	90.00
9	*S. agalacticus*	22.36	90.00
10	*Klebisella spp*	0.00	100.00

One-way analysis of variance
Analysis of Variance for C2

Source	DF	SS	MS	F	P
C1	9	4712	524	1.09	0.391
Error	40	19201	480		
Total	49	23913			

Table 3: Effect Aspirin on diabetic foot infection isolates.

Level	Microorganism	Standard Deviation	Mean
1	*S. aureus*	5.46	94.73
2	*Ps. aeruginosa*	18.59	84.44
3	*St. epidermidis*	12.65	91.68
4	*Klebsiella pneumonia*	28.87	83.33
5	*Proteus mirabilis*	23.57	83.33
6	*Escherichia coli*	17.89	88.00
7	*Proteus vulgaris*	50.00	50.00

One-way analysis of variance
Analysis of variance for C2

Source	DF	SS	MS	F	P
C1	6	6625	1104	1.63	0.176
Error	28	18978	678		
Total	34	25604			

Table 4: Effect of aspirin on UTI (urinary tract infection) isolates.

Level	Microorganism	Standard Deviation	Mean
1	*E. coli*	51.12	56.00
2	*S. aureus*	42.43	70.00
3	*E. faecalis*	42.43	70.00
4	*S. saprophyticus*	28.28	80.00
5	*Proteus*	29.81	20.00
6	*Pseudomonas*	36.51	60.00
7	*S. pyogenes*	50.55	53.33
8	*S. epidermidis*	22.36	90.00
9	*S. agalacticus*	44.72	70.00
10	*Klebisella spp*	54.77	60.00

One-way analysis of variance
Analysis of Variance for C2

Source	DF	SS	MS	F	P
C1	9	15873	1764	1.02	0.441
Error	40	69163	1729		
Total	49	85036			

DISCUSSION

Urinary tract infection is the most common of all bacterial infections. In the present study *E. coli, S .aureus, E. faecalis* and *S .saprophyticus* were mainly isolated from UTI as shown in fig. 1. Other studies also show variability in prevalence of common bacterial pathogens isolated (Fishman and Armstrong 1972).

Bacteria that were mainly isolated from diabetic foot infection were *S. aureus*. *P. aeruginosa, S. epidermidis K. pneumonia* and *P. mirabilis* and *E. coli.* Other studies also show that *S. aureus* and *P. aeruginosa* were the most common causes of diabetic foot infections (Sharma *et al.,* 2006).

Effect of Aspirin on isolates of UTI shows that *S. aureus* and *E. faecalis* indicate 100% inhibition at 100, 500, 1000 µg /ml concentrations. Growth of *S. aureus* with NSAIDS salicylate reduces susceptibility of the organism to multiple antimicrobials. Transcriptome analysis revealed that growth of *S. aureus* with salicylate leads to the induction of genes involved with gluconate and formate metabolism and represses genes required for gluconeogenesis and glycolysis (Muller1 *et al.,* 1998; Polonio *et al.,* 2001). In addition, Aspirin being a weak acid could increase the membrane potential of *E. coli S. typhimurium*, and *P. aeruginosa*, and thereby enhance the susceptibility to novobiocin and aminoglycoside (Bitko *et al.,* 1997). Salicylates can also increase the uptake of divalent ions such as cadmium in *E. coli*, potentiating Cd^{2+} toxicity, independent of an increase in membrane potential (Kopp and Ghosh, 1994; Rainer and Bernhard 2002).

Antibacterial effect of Aspirin on isolates of diabetic foot infection shows that 100 µg/ml concentrations of Aspirin were mostly inhibitory for *S. aureus*, and other isolates.

Aspirin has been used in vitro in a number of experimental models to mitigate the course of disease caused by *S.aureus* **(**James *et al.*, 2007). 87% of *S.epidermidis* was inhibited at 10 µg/ml concentration while 50 µg/ml concentrations show 71.42% inhibition. Other reports indicate that increase in concentration inhibition decreases extra cellular biofilm production by *S. epidermidis* (Aumercier *et al.,* 1990; Cohen *et al.,* 1993). *P. mirabilis* shows 100% inhibition. *E. coli* indicates 60% and 80% inhibition at 10 and 50 µg/ml concentrations while *K. pneumonia was* also susceptible to Aspirin. Previous studies have reported that Aspirin and other NSAIDs interfere with growth of both Gram negative and Gram positive bacteria in vitro (Rosner and Aumercier, 1990).

Effect of Tylenol on isolates of UTI indicates *E. coli* showing 93.33% inhibition. Other studies indicate that Salicylate and antibiotics are often administered simultaneously and subsequent high levels of both drugs can compromise their effectiveness. Salicylate and other membrane permeating weak acids, increase the resistance of *E. coli* to ampicillin, tetracycline, chloramphenicol, nalidixic acid and cephalosporins (Rosner, 1985).

The effect of Tylenol on isolates of diabetic foot infection show that *S. aureus* inhibition increases in dose dependent manner *P.aeruginosa. S. epidermidis, K. pneumoniae* has 100% inhibition. Some strains of *S. epidermidis* secrete mucoid extracellular polymers which are polysaccharides proteins and teichoic acid that promote biofilm formation and become important components of the biofilm matrix. Salicylate can inhibit 95% the production of some of these components (Rosner and Aumercier 1990; Polonio *et al.,* 2001).

Apart from analgesic, anti-pyretic, anti-inflammatory action, Aspirin and Tylenol have marked anti bacterial effect on isolates from UTI and diabetic foot infection. Aspirin interfere with growth of both Gram negative and Gram positive bacteria at 500 µg/ml concentrations while Tylenol interferes more effectively at 100 µg/ml concentration. It is suggested that both drugs can be used with antibiotic for effective treatment.

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