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Efficacy of Anti-Microbial Agents with Ascorbic Acid in Catheter Associated Urinary Tract Infection

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Abstract

Background: Rapid development of microbial resistance is an emerging health care issue of developing countries. In this study we evaluated the susceptibility pattern of bacterial pathogens causing catheters associated urinary tract infection (CAUTI) against commonly prescribed antibiotics alone and in combination with ascorbic acid in institutional settings.

Method: The urine samples from 100 catheterized patients were collected and analyzed for their causative microbial agents. Susceptibility patterns of bacteria against amikacin, ampicillin, amoxicillin/clavulanic acid, ceftriaxone, and ciprofloxacin alone and in combination with ascorbic acid were analysed by Kirby-Bauer disk diffusion method.

Results: The pathogens identified in CAUTI patients were *Candida* spp. (22%), *Citrobacter* (22%), *E. coli* (27%), *Enterobacter* (5%), *S. aureus* (4%). Mixed growth of more than three colony types was observed in 20% cases. Bacteria appeared to be highly resistant against ceftriaxone (84%), followed by amoxicillin/clavulanic acid (83%) and ampicillin (76%). Maximal sensitivities were observed against amikacin (74%) and ciprofloxacin (71%). Improvement in susceptibility pattern of causative microorganisms was observed against Ceftriaxone and ciprofloxacin *In vitro*, when used in combination with ascorbic acid.

Conclusion: The observed synergistic interactions between ceftriaxone/ascorbic acid and ciprofloxacin/ascorbic acid indicate the beneficial aspects of combination chemotherapy by minimizing the microbial resistance and increase the effectiveness of CAUTI treatment.

Keywords: Antimicrobial resistance; CAUTI; Ascorbic acid; *E. coli; Enterobacter; S. aureus*

Introduction

Hospital acquired infection is an infection that occurs in patients within 48 h of hospital admission, 3 days of discharge from hospital or 30 days of an operation [1]. The common type of hospital acquired infections are bloodstream infection eg, ventilator-associated pneumonia, urinary tract infection, and surgical site infection [2]. Hospital-acquired Urinary tract infections are mainly due to catheterization. Catheter associated-bacteriuria is a common health care-associated problem in the world, accounting for up to 60% of nosocomial infections in tertiary care hospitals of developing countries like Pakistan [3]. The prevalence in these countries is greater because of poor hygiene. In hospitals, the risk of emerging catheter associated urinary tract infection (CAUTI) is more in patients who are female, elderly or critically ill [4].

Patient intrinsic factors such as obesity increases the chances of hospital acquired infections and is associated with under dosing of antimicrobial agents in the treatment of hospital acquired infections or prophylactically to obese patients [5]. Postoperative patients are at a higher risk of developing CAUTI because of more than 48 h of catheterization. The use of catheters can result in the entry of bacteria into the bladder [6]. caused by*C. freundii, E. coli, P. mirabilispseudomonas spp.*Gram-negative bacteria that cause CAUTI have a number of factors that are virulent such as adhesion of pathogen, motility of bacteria, formation of bio film, and nutrients availability along with factors that are harmful for the host [8]. Large number of patients is being diagnosed inappropriately with Catheter associated urinary tract infections (CAUTIs), for which they get therapy that is not recommended to them. This unsuitable therapy is potentially harmful because the risk of emergence of resistant pathogens increases [9], super infections or cross-infections increases and unnecessary expenditure on treatment [10].

Ascorbic acid is a sugar acid and have antioxidant properties. Natural antibiotics such as ascorbic acid, garlic and zinc with prescription antibiotics are beneficial and help the body fight against infections, boost up immune system and prevent the disease, without any risk of developing resistance [11]. Current development in the treatment of infections includes antioxidants along with antibiotics. Antioxidants such as ascorbic acid are usually prescribed to restore the immunity and health in the patients [12]. Moreover there is a possibility of potentiating the antibacterial activities of the antibiotic by antioxidants [13].

Several investigators reported that ascorbic acid has inhibitory effect against Gram positive and Gram negative bacteria so, the idea of using these natural antibiotics for the treatment of CAUTI originated due to emerging resistance against the antibiotics currently available. The emergence of multi-drug resistant bacteria and the infectious diseases caused by them is a serious worldwide problem [14]. Therefore combination therapy of antibiotics with natural antibiotics is profitable for patients with serious infections caused by highly resistant bacteria. Life threatening bacterial infections can be treated by administering large doses of ascorbic acid along with antibiotics [15]. The usefulness of Ascorbic acid together with antibiotics is significant, high dose ascorbic acid has been shown to have synergistic effects when used with antibiotics. Ascorbic acid is an antioxidant and it augmented the bactericidal activity of several antibiotics such as ofloxacin, doxycycline and tetracycline [16]. Owing to its antioxidant property, it produces oxidative radical that increases oxidative stress and affects the metabolism of bacteria and these changes result in the inhibition of bacterial growth *In vitro* [17]. *In vitro* studies suggest that ascorbic acid posseses bactericidal activity at concentrations greater than minimum inhibitory concentrations required for antibiotics [18].

The daily intake of ascorbic acid 100 mg plays a vital role in minimizing the chances of urinary tract infections in pregnancy [19]. Several reports have stated that antibacterial effect of levofloxacin is enhanced by ascorbic acid [20]. The use of ascorbic acid provides protection against adhesion by uropathogens within the urinary tract. Therefore, the current study is designed to evaluate the *in-vitro* interactions between ascorbic acid and certain known antibiotics like amoxicillin/clavulanic acid, ampicillin, amikacin, ceftriaxone and ciprofloxacin against *E. coli, Citrobacter, Enterobacter* and *S. aureus*, the causative agents of CAUTI and this study is focused on the increased efficacy of antibiotics with ascorbic acid.

Materials and Methods

The Prospective observational study was performed in patients developed the urinary tract infection during the period of catheterization in Medical units, Gynecology and Obstetrics wards and Urology ward of Tertiary care teaching hospital during the period of 1st March to 31st October 2014.

Catheterized patient suspected to develop pathogenic infection during period of catheterization and appearance of symptoms after 48 h of catheterization.

Midstream urine samples along with catheter tips were collected from 100 patients and processed for bacteriological analysis and susceptibility testing of bacteria against antibiotics in Pathology laboratory, within half an hour after collection. In laboratory, urine specimen was centrifuged at 2,400 Revolution per Minute (RPM) for microscopic examination [21]. Only those urine samples were cultured that had Pus cells and Red blood cells greater than 2 per high power field (pus cells ≥ 2/HPF) as per British Columbia guidelines (BC guidelines).

Bacteriological culture media such as Nutrient agar, CLED agar, Muller Hinton agar No.2, MaConkey agar, Eosin Methyline Blue Agar (EMB), Triple iron agar sugar, manufactured by M/S Merck's Germany, antibiotic disks manufactured by Difco Laboratories, USA were used in the study. The organisms isolated from urine culture were *Candida spp, Citrobacter, E. coli, Enterobacter, S. aureus* and mixed growth of more than 3 colony types. All the bacterial strains were obtained from Pathology/Microbiology laboratory of tertiary care teaching hospital, Lahore Pakistan. The strains isolated from urine culture were grown in the nutrient broth and maintained at nutrient agar slant at 4°C.

All antibiotics manufactured by Difco Laboratories, USA purchased from scientific traders Ltd. (Lahore, Pakistan). Viz amikacin (30 µg/disc), Amoxicillin/Clavulanic acid (30 µg/disc), Ampicillin (10 µg/disc), Ceftriaxone (30 µg/disc), Ciprofloxacin (5 µg/disc), Ascorbic acid (30 µg/disc) (Table 1).

Drugs	Concentration (µg)	Resistant (mm) R	Intermediate Sensitive(mm) IR	sensitive(mm) S
Ampicillin	10 µg	≤ 11	Dec-13	≥ 14
Amoxicillin/ clavulanic acid	30 µg	≤ 13	14-17	≥ 18
Ceftriaxone	30 µg	≤ 14	15-19	≥ 20
Ciprofloxacin	5 µg	≤ 15	16-20	≥ 21
Amikacin	30 µg	≤ 14	15-16	≥ 17

Table 1: Use as a standard for interpretation of susceptibility testing results (NCCL).

Combination discs of ascorbic acid with these 5 antibiotics were prepared by making stock solution of antibiotic individually and ascorbic acid stock solution. Impregnated these solutions on 6 mm filter paper discs by micropipette and dry them and store in sterile air tight labeled containers [22]. Antibacterial susceptibility of antibiotics alone and in combination with ascorbic acid was assessed against *Citrobacter, E. coli, Enterobacter and S. aureus.* by agar disc diffusion method [23]. The petri-dishes were prepared by pouring 20 ml of molten Muller Hinton Agar (MHA) in biological safety cabinet class-2 type to avoid extraneous contamination. The MHA was seeded with 200 µl test culture containing 1×10^8 cfu/ml as McFarland 0.5 turbidity standard [24].

Petri dishes were allowed to solidify. Antibiotic discs and combination discs prepared were placed on the surface of the agar dishes which had previously been inoculated with tested microorganisms. All petri-dishes were incubated at 37°C for 24 h. Results were recorded by measuring zone of inhibition around discs. All the tests were performed in triplicate and the mean values are recorded. Dimethyl Sulfoxide (DMSO) was used as negative control. The antibacterial activity was expressed as mean zone of inhibition diameters (mm).

All experiments were repeated three times. SPSS version 16 was used for statistical analysis. One-way ANOVA was used for comparing the antibiotics and antibiotic ascorbic acid combination in different bacteria. For ANOVA two groups of antibiotics were made on the basis of their standards for sensitivity interpretation, Group-A included ampicillin and amikacin because their standards for sensitivity interpretation were ≥ 14 and ≥ 17 respectively. Combination of Ascorbic acid with Ampicillin and Amikacin was also analysed in this group. Group-B had Amoxicillin/Clavulanic acid, Ceftriaxone and Ciprofloxacin because their standards for sensitivity interpretation were ≥ 18, ≥ 20 and ≥ 21 respectively. Analysis of these drugs combination with ascorbic acid was also made in Group B. P-value less then ($P<0.05$) 0.05 was taken as significant.

Results

Total 100 urine samples of catheterized patients from medical, Gynaecology and Urology wards of Tertiary care teaching hospital were collected and processed for bacteriological analysis. 22 (%) urine samples contained *Citrobacter spp.* 22 (%) were *Candida spp.* 27 (%) were *E. coli* 5(%) were *Enterobacter* and 4 (%) were *S. aureus*, and 20 (%) were mixed growth of more than 3 colony types of bacteria.

To check the susceptibility of uropathogens against available antibiotics and their combination with ascorbic acid, only those 58 patients were included in the study that had *Citrobacter, E. coli, Enterobacter and S. aureus* because this study was aimed for susceptibility of bacteria to antibiotics so patients that had candida spp. and mixed growth of more than 3 colony types were not included.

The efficacy of antibiotics such as Amikacin, Ampicillin, Amoxicillin/Clavulanic Acid, Ceftriaxone and Ciprofloxacin and ascorbic acid were checked against isolated uropathogens by Kirby Bauer disc diffusion method. It was observed that uropathogens were 84% resistant to ceftriaxone and 83% resistant to amoxicillin/clavulanic acid. Uropathogens were sensitive to Amikacin and ciprofloxacin with their sensitivities 74% and 71% respectively (Table 2).

Bacteria	Monodrug				Combination with Ascorbic Acid		
	Drugs	R cases	IS cases	S cases	R cases	IS cases	S cases
E. coli	Amoxicillin/Clavulanic acid	74%	0	26%	48%	26%	26%
	Amikacin	7%	4%	89%	7%	4%	89%
	Ceftriaxone	78%	11%	11%	33%	44%	22%
	Ciprofloxacin	15%	19%	66%	0	15%	85%
	Ampicillin	70%	0	30%	70%	0	30%
Citrobacter	Amoxicillin/Clavulanic acid	86%	0	14%	86%	0	14%
	Amikacin	18%	5%	77%	91%	9%	0
	Ceftriaxone	91%	0	9%	14%	50%	36%
	Ciprofloxacin	18%	14%	68%	0	5%	95%
	Ampicillin	86%	5%	9%	86%	5%	9%
Enterobacter	Amoxicillin/Clavulanic acid	80%	0	20%	80%	0	20%
	Amikacin	40%	0	60%	20%	20%	60%
	Ceftriaxone	80%	0	20%	0	80%	20%
	Ciprofloxacin	40%	0	60%	0	0	100%
	Ampicillin	80%	0	20%	80%	0	20%
S. aureus	Amoxicillin/Clavulanic acid	100%	0	0	100%	0	0
	Amikacin	50%	0	50%	0	25%	75%
	Ceftriaxone	100%	0	0	0	100%	0
	Ciprofloxacin	50%	25%	25%	0	25%	75%
	Ampicillin	100%	0	0	100%	0	0

Table 2: Sensitivity pattern of Bacteria against monodrug and combination with a. acid.

It was observed that the resistant cases of bacteria were decreased when antibiotic and ascorbic acid combination disc was used. The significant results (P=0.00<0.05) were observed with ceftriaxone/ascorbic acid and ciprofloxacin/ascorbic acid combination. It was observed that the resistance of Citrobacter was increased when amikacin/ascorbic acid combination was used.

It was observed that when antibiotics alone and combination with ascorbic acid were checked by disc diffusion method a significant increase in mean zone of inhibition diameters were observed (Table 3).

Discussion

CAUTI is common hospital acquired infection that develops as a result of catheterization and occurs in approximately two patients per

hundred admissions. In short term catheterization, common bacteriuric species *are Citrobacter spp. Candida spp. E. coli.* Mixed growth *Citrobacter spp. Candida spp. E. coli* mostly occurs in long term catheterized patients [25].

In *in-vitro* studies, the susceptibility testing of uropathogens against antibiotics alone and their combination with ascorbic acid was checked by disc diffusion method. Combination discs of antibiotics and ascorbic acid were prepared and their efficacy against isolated pathogens was checked by measuring their zone of inhibition around disks. Synergistic effects were produced when antibiotics and ascorbic acid use together. It was observed that the activity of antibiotics was greatly enhanced and the resistance of bacteria was reversed with ascorbic acid. Total 27 samples of *E. coli* were checked for their susceptibility against antibiotics and antibiotic^{+} ascorbic acid combination. It has been observed that ascorbic acid increases the zone diameters of amoxicillin/clavulanic acid but did not turn into sensitive against *E. coli.*

E. coli was resistant against ceftriaxone and 21 cases appeared resistant but when it was used in combination with ascorbic acid, the resistant cases turned into intermediately sensitive and sensitive cases so significant results were obtained. With ciprofloxacin, the number of sensitive cases of *E. coli* was greater and its combination with ascorbic acid further enhanced the number of sensitive cases (Table 3). 22 cases of *Citrobacter* were analysed for their sensitivity pattern against antibiotics alone and along with ascorbic acid combination. *Citrobacter* was resistant against amoxicillin/clavulanic acid, ceftriaxone and ampicillin. The efficacy of amikacin and ciprofloxacin was greater than the rest of the drugs. amikacin when used in combination with ascorbic acid, number of sensitive cases of *Citrobacter* reduced and resistant cases increased (Table 3).

Bacteria	Drugs	Monodrug	Combination With Ascorbic Acid
		Mean zone of inhibition diameter (mm)	
E. coli	Amoxicillin/Clavulanic acid	19	20.71
	Amikacin	19.83	23.74
	Ceftriaxone	23.5	30.5
	Ciprofloxacin	22.94	30.61
	Ampicillin	15.38	15.75
Citrobacter	Amoxicillin/Clavulanic acid	18.33	17.66
	Amikacin	20.6	13
	Ceftriaxone	21.5	32
	Ciprofloxacin	22.86	31.75

Table 3: Mean zone of inhibition diameters of antibiotics alone and in combination with ascorbic acid.

Average zone of inhibition diameters were decreased in amikacin ascorbic acid combination as compared to amikacin alone. There was no change in average zone of inhibition diameters of Ampicillin alone and Ampicillin ascorbic acid combination (Figure 1). Ciprofloxacin and Ceftriaxone average zone of inhibition diameters were increased when used in combination with Ascorbic acid (Table 3). The result from current study revealed that ascorbic acid somehow improves the susceptibility of ceftriaxone and a noticeable increase in the zone of inhibition was observed with ciprofloxacin and ceftriaxone. Studies suggest that ascorbic acid inhibits the growth of *E. coli* by inhibiting the uptake of sugars through interaction with membrane components such as phosphotransferase system; transport system is sensitive to ascorbate and is reversibly blocked by ascorbic acid [26].

However, ascorbic acid has no effect on the growth of *Citrobacter* [27] that is why when given with Amikacin, it hindered the transport of Amikacin into bacteria and the zone of inhibition was smaller when amikacin was given with ascorbic acid. The reason is Amikacin is basic in nature & acidic pH of ascorbic acid impairs Aminoglycosides transport into bacteria probably as a result of its large ionization at pH 5.4 *versus* pH 7.4. In the presence of acidic medium, the activity of Aminoglycosides decreases because Minimum Inhibitory Concentration (MIC) values increases in acidic P^{H} [28].

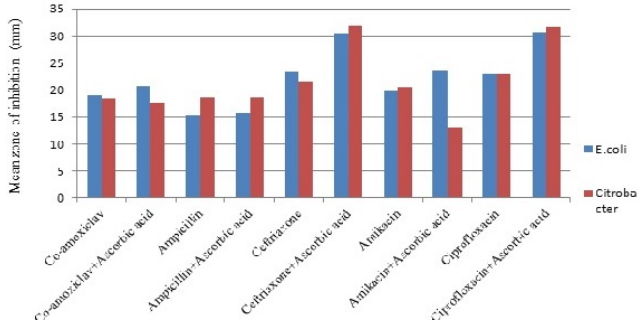

Figure 1: Graph showing changes in zone of inhibition diameters of antibiotic alone and in combination with ascorbic acid of *E. coli* and *Citrobacter.*

Moreover, the growth of *S. aureus* was found to be inhibited when ascorbic acid was added, perhaps due to increases in the oxidative stress on bacterial cells [29]. The inhibitory effect produced by ascorbic acid may be due to its anti-quorum sensing activity and it is considered as quorum sensing inhibitor [30]. Quorum sensing also known as cell to cell signalling produces as a result of large population of bacteria and is responsible to produce greater virulence of bacteria resulting in the formation of biofilm and toxins production [31]. The combination of levofloxacin and ascorbic acid produces inhibitory effect of the microbial adherence to the catheter with the complete elimination of bio film formation [32] and in this way ascorbic acid/levofloxacin exerts its inhibitory effect. In *In vitro* study, it was observed that ascorbic acid enhanced the activity of antibiotics against isolated uropathogens when used in combination with antibiotics. Substances that enhance the activity of antibiotics or reverse the antibiotic resistance are classified as modifiers of antibiotic activity [33] and this study suggests that ascorbic acid is antibiotic modifier. Ascorbic acid supplementation with antibiotics provides protection against adhesion by uropathogens and colonization within the urinary tract and this agrees with the results obtained from the present study [33,34].

Conclusion

It has been found from study that ascorbic acid is a synergistic enhancer for several antibiotics particularly for Ceftriaxone and

Ciprofloxacin. Combinations of antibiotics with ascorbic acid demonstrate an in-vitro synergistic effect and increase the effectiveness of therapy against resistant bacterial strains. Study showed bacterial strains appeared to be resistant with ceftriaxone but the resistance was reversed with ascorbic acid/ceftriaxone. Ascorbic acid improves the efficacy of ceftriaxone and the effectiveness of ciprofloxacin was also enhanced with ascorbic acid *In vitro*. The observed antimicrobial efficacy and synergistic interactions between ceftriaxone/ascorbic acid and ciprofloxacin/ascorbic acid combination gives promising approach for its use in CAUTI patients and studies suggest that ascorbic acid is an antioxidant and it may be used in the treatment of infections with antibiotics such as Ceftriaxone and ciprofloxacin. Ascorbic acid also decreases the usage of antibiotics needed to inhibit or kill bacteria so it has been suggested from study that combination therapy of antibiotics/ascorbic acid could be effective in the treatment of CAUTI and useful in fighting against resistant strains of bacteria however further *in-vivo* experiments are needed to confirm the bacterial protection with this combination.

Competing Interest

The authors declare that they have no competing interest.

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Transparency Declaration

None to declare by all authors. It's the source of mutual interests for all authors.

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