ORIGINAL RESEARCH ARTICLE

Bacteriological Analysis of Asymptomatic Bacteriuria in Diabetic Individuals from Rural Area

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Abstract:

Asymptomatic bacteriuria (ASB) is common in diabetic individuals because of decreased antibacterial activity of the urine as a result of dilution of inhibitory substances. The present study was carried out to find out the causative agents associated with asymptomatic bacteriuria and their antimicrobial susceptibility pattern to institute the rational antibiotic therapy to avoid further morbidity and life threatening complications in diabetic individuals from rural area. A total of 212 urine samples from diabetic individuals and 212 from non-diabetic individuals were processed for identification and antimicrobial susceptibility testing. Of the 212 samples from diabetic individuals, 35 (16.50%) were found positive for asymptomatic bacteriuria (ASB) and among the non-diabetic individuals, seven (3.30%) were positive for ASB. In diabetic individuals, *E. coli* (54.28%) and in non-diabetic individuals, *Klebsiella* (57.14%) were found to be the most common bacterial isolates. In both the groups imipenem (97.14%) was found to be the most effective antimicrobial agents. It is concluded from the findings that routine screening of asymptomatic bacteriuria in diabetic individuals to initiate rational antibiotic therapy and to avoid further serious complications is of utmost importance.

Keywords:

Asymptomatic bacteriuria, Diabetes, Bacterial pathogens, Antimicrobial resistance

How to cite this article: Megha Rastogi, Milind Davane and Basavraj Nagoba . Bacteriological Analysis of Asymptomatic Bacteriuria in Diabetic Individuals from Rural Area. Walawalkar International Medical Journal 2018; 5(2):1-10. http://www.wimjournal.com

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Received date: 17/10/2018 Revised date: 23/10/2018 Accepted date: 27/12/2017
DOI Link:

Introduction:

Symptomless infections of urinary tract are known as covert or asymptomatic bacteriuria (ASB). ASB is a common condition, especially in diabetic individuals because of decreased antibacterial activity of the urine as a result of dilution of inhibitory substances, defects in polymorphonuclear leukocyte function or increased adhesive capacity of bladder epithelial cells. The diabetic individuals are more prone to symptomatic and asymptomatic urinary tract infection (UTI). A three-fold higher prevalence rate in diabetic women and men than non- diabetic women and men has been reported. The reported global prevalence rates of ASB in diabetic females ranges from 9 - 29% whereas in diabetic males it ranges from 0.7-11%.[1-3] The morbidity of ASB in diabetic individuals may include either short-term risk of developing a symptomatic UTI and its more serious complications or the long-term risks of developing serious diabetic complications.

The increased prevalence of ASB in recent years has been found in diabetic individuals. It has also been found that bacterial species commonly associated with ASB exhibit resistance to commonly used antimicrobial agents raising the questions regarding future clinical reliability of some conventional antimicrobial agents for the treatment of ASB. These findings show necessity of urine culture and sensitivity to initiate rational antibiotic therapy and to assess the results of treatment.[4,5]

In the present study an attempt has been made to study the pattern of ASB in diabetic individuals from rural area, to find out the various causative agents associated with ASB and their antimicrobial susceptibility pattern to institute the rational antibiotic therapy to avoid further morbidity and life threatening complications in diabetic individuals from rural area.

Methods:

The study protocol was approved by institutional ethics committee. A total of 212 urine samples from diabetic individuals and 212 from non-diabetic individuals attending a tertiary care hospital in rural area were collected by clean catch method in a sterile container. Specimens were transported immediately to microbiology laboratory for further processing.

The specimens were inoculated on Blood Agar and Mac-Conkey agar. A calibrated loop (with an internal diameter of three mm delivering 0.001ml of urine) was used. A loopful of urine was inoculated and plates were incubated at 37˚C for 18-24 hours. After incubation, colonies were counted on blood agar plate and the number of bacteria present in urine was calculated by multiplying number of colonies by 1000. Specimens of urine showing counts > 10^5 CFU/ml were considered as significant bacteriuria.

The morphology of each different type of colony was noted and each colony was studied for gram reaction and colony morphology, and processed further for identification using standard procedures.[6,7] Each isolate was subjected to the study of antimicrobial susceptibility pattern using Kirby-Bauer's disc diffusion method against Amikacin (30µg), Ceftazidime (30µg), Gentamicin (30µg), Ciprofloxacin (5µg), Tetracycline (30µg), Nitrofurantoin (300µg), Norfloxacin (10µg), Nalidixic acid (30µg), Imipenem (10µg), Meropenem (10µg) and Cefoxitin (30µg).[8] The organism was reported as susceptible or resistant according to the guidelines of clinical and laboratory standards institute (CLSI).[9] Nalidixic acid and nitrofurantoin were used against gram-negative bacteria only. However, cefoxitin and tetracycline were used against gram-positive bacteria only. Statistical analysis was carried out by using appropriate statistical tests.

Results:

Of the 212 samples from diabetic individuals, 35 (16.50%) were found positive for ASB and among the non-diabetic individuals, seven (3.30%) were positive for ASB. In diabetic individuals, *E. coli* (54.28%) was found to be the most common bacterial isolate followed by *Klebsiella* spp. (22.85%), *Citrobacter* spp. (11.42%), *Pseudomonas aeruginosa* (8.57%) and Micrococci (2.85%) (Table 1).

Table 1: Bacteria associated with ASB in a study group.

Name of bacteria	No. of isolates
E. coli	19 (54.28%)
Klebsiella spp.	8 (22.85%)
Citrobacter spp.	4 (11.42%)
Pseudomonas aeruginosa	3 (8.57%)
Micrococci	1 (2.85%)
Total	**35 (99.97%)**

In non-diabetic individuals, *Klebsiella* spp. (57.14%) was found to be the most common bacterial isolate followed by *Pseudomonas aeruginosa* (28.57%) and *E. coli* (14.28%) (Table 2).

Table 2: Bacteria associated with ASB in non-diabetic individuals

Name of bacteria	No. of isolates
Klebsiella spp.	4 (57.14%)
Pseudomonas aeruginosa	2 (28.57%)
E. coli	1 (14.28%)
Total	**7 (99.99%)**

The results of antimicrobial susceptibility testing of bacterial isolates of ASB from diabetic individuals showed imipenem (97.14%) as most effective agent followed by amikacin (82.85%), nitrofurantoin (65.71%), gentamicin (60%), meropenem (45.71%), ciprofloxacin (42.85%), ceftazidime (40%), norfloxacin (34.28%), nalidixic acid (25.71%), cefoxitin and tetracycline (2.85% each) (Table 3).

Table 3: Antibiogram of Bacterial Isolates from Study Group

Name of the bacteria	No. of isolates	Antimicrobial agents (No. and percentage)										
		AK	NIT	NOR	NA	G	CIP	CN	T	I	MR	CAZ
E. coli	19	17 (89.47)	14 (73.68)	6 (31.57)	8 (42.10)	12 (63.15)	8 (42.10)	-	-	19 (100)	8 (42.10)	9 (47.36)
***Klebsiella* spp.**	8	4 (50)	6 (75)	2 (25)	00	3 (37.5)	2 (25)	-	-	8 (100)	3 (37.5)	2 (25)
***Citrobacter* spp.**	4	4 (100)	3 (75)	3 (75)	00	3 (75)	4 (100)	-	-	3 (75)	3 (75)	3 (75)
Pseudomonas aeruginosa	3	3 (100)	00	00	00	2 (66.66)	00	-	-	3 (100)	1 (33.33)	00
Micrococci	1	1 (100)	00	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	00
Total	35	29 (82.85)	23 (65.71)	12 (34.28)	9 (25.71)	21 (60)	15 (42.85)	1 (2.85)	1 (2.85)	34 (97.14)	16 (45.71)	14 (40)

Note: Figures in parentheses indicate percentage

The results of antimicrobial susceptibility testing of bacterial isolates of ASB from non-diabetic individuals also showed imipenem (100%) as most effective agent followed by amikacin and gentamicin (71.42% each), meropenem (57.14%), nitrofurantoin, norfloxacin, ciprofloxacin and ceftazidime (42.85% each) (Table 4).

Table 4: Antibiogram of bacterial Isolates of ASB from Non-diabetic individuals

Name of the bacteria	No. of Isolates	Antimicrobial agents (No. and percentage)										
		AK	NIT	NOR	NA	G	CIP	CN	T	I	MR	CAZ
***Klebsiella* spp.**	4	2 (50)	2 (50)	1 (25)	00	2 (50)	1 (25)	-	-	4 (100)	2 (25)	00
Pseudomonas aeruginosa	2	2 (100)	00	2 (100)	00	2 (100)	2 (100)	-	-	2 (100)	2 (100)	2 (100)
E. coli	1	1 (100)	1 (100)	00	00	1 (100)	00	-	-	1 (100)	00	1 (100)
Total	7	5 (71.42)	3 (42.85)	3 (42.85)	00 (00)	5 (71.42)	3 (42.85)	-	-	7 (100)	4 (57.14)	3 (42.85)

Note: Figures in parentheses indicate percentage

Discussion:

The association of diabetes mellitus and UTI is increasingly being reported. ASB is common among diabetic patients and may lead to serious complications, if not properly managed.

A total of 35 cases (16.50%) out of 212 diabetic individuals and seven cases (3.30%) out of 212 non-diabetic individuals were positive for ASB. In diabetic individuals, *E. coli* (54.28%) was the most common bacterial isolate. In majority of the earlier studies *E. coli* has been reported to be the most common bacterial isolate associated with ASB in diabetic individuals.[8-16] The findings of isolation of *E. coli* as the most common isolate are consistent with these earlier reports. The findings of *Klebsiella* spp. as the second most common isolate associated with ASB is also quite similar to most earlier studies.[11,16-19,22] In non-diabetic individuals, *Klebsiella* spp. (57.14%) was found to be the most common bacterial isolate. The findings of *Klebsiella* spp. as the most common bacterial isolate associated with ASB is similar to Odetoyin *et al.* (2008) only.[23] Otherwise these results are in contrast to most of the earlier studies in which either *E. coli* or coagulase negative staphylococci have been reported to be more common agents associated with ASB.[8,10,14,24,25] These results show that the trend is not consistent and it keeps on changing and it varies from study-to-study from different geographical locations.

The imipenem (97.14%) was found to be the most effective antibacterial agent against the isolates of ASB in diabetic individuals. In general, review of earlier studies show that different workers using different antimicrobial agents have reported different patterns of susceptibility/resistance in bacterial isolates of ASB. There is no consistency in the choice of antimicrobial agents and also in the results of different studies from different countries, places and hospitals. Imipenem has been included for susceptibility testing in recent studies only; hence, a few reports are available for comparison. Imipenem has been reported highly effective antimicrobial agent in most of the earlier studies.[22,26-28] Our results are in concurrence with the fact that imipenem is the most effective antimicrobial agent in present situation. Amikacin has been reported to be effective against bacterial isolates of ASB.[16,18,27] The results showing 82.85% susceptibility in bacterial isolates associated with ASB are fairly comparable to these earlier findings. In some of the earlier studies gentamicin and nitrofurantoin have been reported to be more effective antimicrobial agents,[5,18,20,27] but in our study gentamicin was found effective against 60% of bacterial isolates and nitrofurantoin was found effective against 65.71% of isolates only. In some earlier studies, ciprofloxacin has been reported to be more effective antimicrobial agent,[13,14,16] but in our study it was found effective against 42.85% of isolates only. Although meropenem has been reported to be most effective (upto 100% susceptibility) antimicrobial agent in earlier studies,[18,28] it was found effective against 45.71% of isolates only. Other antimicrobial agents like ceftazidime, norfloxacin and nalidixic acid although have been reported to be effective in one or other earlier studies,[5,13,14,16,20] resistance to these agents have been reported more commonly in most of the studies in recent past.[15,19,23] The results show that these agents which are in common use since many years are losing their efficacy because of development of resistance in bacterial isolates commonly associated with ASB.

The results of antimicrobial susceptibility testing of bacterial isolates of ASB from non-diabetic individuals also showed imipenem (100%) as most effective agent. The antimicrobial susceptibility pattern of bacterial isolates associated with ASB in non-diabetic individuals has been rarely studied and reported.[21,24,25] The findings of our study show that there is a major variation in the results of susceptibility pattern of our study and most of the earlier studies. These findings indicate that susceptibility pattern

changes from hospital-to-hospital, population-to-population and location-to-location. It also indicates the importance of study of susceptibility pattern in institution of rational antibiotic therapy, as emphasized by various international authorities that every hospital should have its own antibiotic policy based on antimicrobial susceptibility pattern to decide the treatment strategy, as the standard antimicrobial susceptibility pattern may not hold true for every hospital/area/region.

Conclusion:

The results of our study indicate the importance of periodic screening of asymptomatic subjects for ASB, especially in diabetic females, and frequent follow up in positive cases to prevent development of symptomatic urinary tract infections (UTI) and to avoid various complications of UTI. The increased isolation of bacteria resistant to commonly used antimicrobial agents indicate the importance of susceptibility studies in institution of rational antibiotic therapy and suggests to avoid empirical/blind drug therapy to avoid further complications.

Source of Funding: None to declare

Conflict of Interest – None to Declare

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