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Original Article

Pregnancy-associated asymptomatic bacteriuria and antibiotic resistance in the Maternity and Children's Hospital, Arar, Saudi Arabia

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Abstract

Introduction: The Ministry of Health in Saudi Arabia provides comprehensive antenatal care for all pregnant women with all required investigations. However, it does not include urine culture for diagnosis of asymptomatic bacteriuria (ASB). This is the first study to evaluate the prevalence of ASB among pregnant females, identify the causative organisms and determine their antibiotic susceptibility patterns in the Maternity and Children's Hospital, Arar, Saudi Arabia.

Methodology: This cross-sectional study included 400 pregnant women attending an antenatal clinic. Two midstream urine samples were aseptically collected and screened using standard microbiological techniques including microscopic examination, dipstick testing, and urine culture. In order to interpret the urine culture results, $\geq 10^5$ CFUs/mL was considered significant bacteriuria. Identification of the isolates and their antibiotic sensitivity testing was performed using the Vitek 2 system (BioMérieux, Marcy l'Etoile, France) with the available test kits.

Results: The prevalence of ASB was 8.25% (35/400). Significant positive correlations ($p < 0.05$) were detected between positive urine culture results and random blood sugar, leucocytes, nitrites, pus cells, urine red blood cells, epithelial cells, and mucus. *Escherichia coli* was the most common causative organism (45.7%), followed by *Staphylococcus aureus* (22.9%). *Klebsiella pneumoniae* represented 11.4% of the isolates. Most of the isolated Gram-positive organisms were sensitive to many of the tested antibiotics; most of the detected Gram-negative isolates were resistant.

Conclusions: ASB caused by antibiotic resistant organisms is alarming. Screening for ASB during pregnancy using urine culture and sensitivity testing is of vital importance to improve the maternal and neonatal outcome.

Key words: antibiotic sensitivity; *E. coli*; *S. aureus*; urine culture; UTI; Vitek.

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Introduction

Asymptomatic bacteriuria (ASB) is defined as the presence of a significant number of bacteria in the properly collected urine of a patient along with the absence of typical symptoms or signs of a lower or upper urinary tract infection (UTI). While the etiology of ASB has not been conclusively determined, it is more common among females than among males, possibly due to the shorter female urethra. In the elderly, incomplete bladder emptying contributes to increased incidence of ASB [1].

The prevalence of ASB during pregnancy varies between 33.3 % [2] and 1.7% [3]. There are many factors that affect the incidence of ASB among pregnant women, including age, socioeconomic level, medical or surgical interventions, comorbidities, personal hygiene, and gravidity/parity. The etiology of ASB comprises a wide range of different bacteria including *Escherichia coli* (*E. coli*), *Klebsiella* species, *Staphylococcus aureus* (*S. aureus*), *Streptococcus agalactiae* (*S. agalactiae*), and *Candida albicans* (*C. albicans*) [4].

Pregnant women who have asymptomatic bacteriuria during early pregnancy and are left untreated are at higher risk of developing pyelonephritis, renal failure, preeclampsia, bacteremia, septicemia, and septic shock. In addition, ASB may lead to serious fetal complications, such as intrauterine growth retardation, prematurity, and acute respiratory distress [5-7]. Early diagnosis and treatment of ASB can drastically reduce the incidence of pyelonephritis and prevent preterm labor by up to 20%. The management and screening of UTIs during pregnancy is standard healthcare in high-income countries and is also recommended by the World Health Organization (WHO) for low-middle-income countries [8].

The diagnostic criteria for ASB vary depending on the method of urine samples collection. For example, according to the Infectious Diseases Society of America, diagnosis of ASB for women is made by urine culture of properly collected clean-catch or a catheterized specimen according to one of the following criteria; 1) two consecutive midstream clean catch urine specimens with detection of the same bacterial isolates with $\geq 10^5$ colony-forming units (CFUs) per mL of urine, or 2) a single catheterized urine specimen with detection of the same bacterial isolates with $\geq 10^2$ CFUs per mL of urine [1].

The Ministry of Health (MOH) in Saudi Arabia provides comprehensive antenatal care for all pregnant women with all the required investigations, including urine analysis. However, it does not include urine culture for diagnosis of ASB. Thus, this study is proposed to determine the prevalence of ASB among pregnant women attending the Maternity and Children's Hospital in Arar, Northern Borders, Saudi Arabia, during the antenatal care period. In addition, it aimed to identify the uropathogens and their antibiotic sensitivity profiles.

Methodology

Study design

This cross-sectional study included 400 pregnant women attending the antenatal clinic in Arar Maternal and Children's Hospital, Northern Borders, Saudi Arabia, for regular follow-up. The sample size was calculated according to calculator.net (https://www.calculator.net/sample-size-calculator.html) based on confidence level of 95%, margin error 5%, with a total population of 300,000. Inclusion criteria were pregnant females, aged between 15-45 years. Exclusion criteria were any pregnant female receiving antibiotics or complaining of UTI within 1 month before her hospital visit

Ethical consideration

The study conforms to the ethical guidelines of the 1975 Declaration of Helsinki. Ethical approval to conduct this study was obtained from the Local Committee for Bioethics Northern Border's Ministry of Health, Saudi Arabia (H-09-A-51). All participants signed informed written consents.

Samples collection

Clean catch mid-stream urine specimens were collected by instructing the participants to clean the periurethral area (labial folds, vulva) with soap and water, and to void into a bedpan, urinal, or toilet. Without stopping the urine stream, the sterile collection container was moved into the stream, collecting about 10 mL (the midstream portion of urine) then capped securely. The container was labeled with the patient's name and file number, date, name of the test, and type of specimen and sent to the microbiology laboratory immediately. A second clean catch mid-stream urine specimen was collected from significant positive urine culture subjects for confirmation. Standard microbiological techniques were used.

Microscopic examination

Direct microscopic examination of urine for pus cells was considered positive if pus cells were > 5 per high-power field (HPF). Dipstick tests were carried out using Comber 10 reagent test strips (Analyticon, Lichtenfels, Germany) to detect protein, blood, nitrite, and leukocyte esterase in urine [9].

Urine culture

All urine samples (first and second samples for positive cultures) were cultured using cysteine-lactose-electrolyte-deficient agar (CLED) media. The standard loop technique was used for colony counting where 0.001 mL of urine was inoculated and streaked across the culture plate using the standard sterile loop. The inoculated plates were incubated aerobically at 37 °C for 48 hours.

Interpretation of urine culture

After incubation, the number of colonies was counted. The significance of a positive urine culture was most reliably assessed in terms of the number of colony-forming units (CFUs; viable bacteria) present in the urine. In order to interpret midstream clean catch urine culture results, $\geq 10^5$ CFUs/mL was considered significant bacteriuria denoting certain UTI [10].

Identification of the isolates

The isolates were identified by Gram staining using a commercially available kit and Vitek 2 system, (BioMérieux, Marcy l'Etoile, France), which uses a fluorogenic methodology for organism identification with the available test kits that include ID-GN (Gram-negative bacilli identification) and ID-GP (Gram-positive cocci identification). The Vitek 2 ID-GN card identifies 154 species of Enterobacteriaceae and a select group of glucose-non-fermenting Gram-negative organisms within 10 hours. The Vitek 2 ID-GP card identifies 124 species of staphylococci, streptococci, enterococci, and a select group of Gram-positive organisms within ≤ 8 hours.

Antibiotic sensitivity testing (AST)

All significant urine cultures were subjected to AST using Vitek 2 system which uses a turbidimetric method for susceptibility testing using a 64-well card that is barcoded with information on card type, expiration date, lot number, and unique card identification number. The available test kits include AST-GN (Gram-negative susceptibility) and AST-GP (Gram-positive susceptibility). The Vitek 2 AST are for the most clinically significant aerobic Gram-negative bacilli, *Staphylococcus* spp., *Enterococcus* spp., and *S. agalactiae*. Susceptibility results are available for bacteria in less than 18 hours. Not all antibiotics were reported but only antibiotics that can be safely given during pregnancy were included.

Data analysis

Descriptive statistics were presented as frequency (numbers; n, and percentage) for qualitative variables and mean ± standard deviation (SD) for quantitative variables. Spearman's rank correlation coefficient (r) was used to examine the correlation between the urine culture results and the other studied parameters. The p value ≤ 0.05 was considered as statistically significant. The Statistical Package for Social Sciences (SPSS) software version 20 was used for data entry and analysis.

Results

A total of 400 pregnant women attending the antenatal clinic in Arar, Northern Borders, Saudi Arabia were enrolled in this cross-sectional study. Table 1 presents the demographic data and its association with the results of the urine culture. The age of the studied women ranged from 15 to 42 years (26.95 ± 6.16 SD) and body mass index (BMI) ranged from 16.03 to 49.0 (28.935 ± 6.25). The mean value of random blood sugar (RBS) was 97.83 ± 23.78 mg/dL with a range of 64-200 mg/dL. Their gravidity ranged from 1 to 13 times while the parity range was 0 to 8 times. Significant positive correlation was detected between positive urine culture findings and RBS ($p < 0.001$ / r = 0.167). However, no significant correlations were detected between positive urine culture and each of the other characteristics of the participants.

The frequency of pregnancy duration was compared with associated diseases, and blood pressure among the studied subjects. A total of 130 (32.5%) subjects were in their first trimester, 124 (31.0%) were in the second trimester and 146 (36.5 %) were in the third trimester. With regards to the associated diseases, 11 (2.5%) subjects had gestational diabetes, 6 (1.5%) diabetes mellitus (DM), and 10 (2.25%) suffered hypothyroidism, while most of the remaining studied subjects had no diseases. 378 (94.5%) subjects were normotensive, and 22 (5.5%) subjects were hypertensive. A significant positive correlation was detected between positive urine culture results and RBS ($p < 0.001$). No significant correlations were detected between urine culture and pregnancy duration, gravidity and parity, blood pressure, or associated diseases ($p > 0.05$).

The urine physical and chemical examination characteristics were analyzed. Urine protein was negative in 324 (81.0%) of the cases and positive (+++) in only 4 (1.0%) of the subjects. With regard to ketones, 360 (90.0%) subjects presented with negative ketones and 4 (1.0%) were positive (+++). Urine glucose was absent in 392 (98.0%) and positive (++) in only 1 (0.25%) of the subjects. Blood was absent in 349 (87.25%), and positive (+++) in 7 (1.75%) of the subjects. Leucocytes were negative in 299 (74.75%),

Table 1. Demographic, anthropometric and pregnancy-related characteristics of women investigated for asymptomatic bacteriuria and correlation with the results of urine culture. Data shown are frequencies, range, mean ± SD, and results of correlation analysis; r and p values.

Variables	Range	Mean ± SD	r	p
Age (years)	15-42	28.95 ± 6.16	-0.028	0.578
BMI	16.03-49	28.93 ± 6.25	0.075	0.135
RBS (mg/dL)	64-200	97.83 ±23.78	0.167	0.001*
Gravidity (n)	1-13	3.55 ± 2.47	0.050	0.321
Parity (n)	0-8	2.02 ± 1.88	0.061	0.227

BMI: body mass index, RBS: random blood sugar, * significant positive correlation.

and (+) in 47 (11.75%), (++) in 30 (7.5%), (+++) in 11 (2.75%) and (++++) in 13 (3.25%) of the subjects. Nitrites were not detected in 371 (92.75%) and positive (+++) in 5 (1.25%) of the subjects. Significant positive correlations were detected between positive urine culture results and urine leucocytes ($p < 0.0001$ / r = 0.371), and urine nitrites ($p < 0.0001$ / r = 0.304).

The urine microscopic examination findings revealed the presence of pus cells at less than 5/HPF among 307 (76.75%) subjects. Similarly, more than 5 RBCs/HPF were seen in 3.25% of subjects. As regards the epithelial cells, they were also detected in most cases (357; 89.25%) at different concentrations. Mucus was not detected in 147 (36.75%) and was positive in 253 (63.0%) of the cases. Bacteria were absent in 28 (7.0%) and positive in 21 (5.25%) of the subjects. Significant positive correlations were detected between positive urine culture results and urine pus cells (p 0.022 / r = 0.115), urine RBCs (p 0.0001 / r = 0.209), urine epithelial cells (p 0.004 / r = 0.143) and urine mucus (p 0.002 / r = 0.158).

Out of the 400 urine specimens tested 219 (54.75%) cultures were negative without any bacterial growth, while non-significant growth was recorded in 146 (36.5%) samples. Significant bacteriuria was detected in 35 (8.75%) of the subjects.

As regards the isolated organisms, *E. coli* was the most common causative organism detected (45.7%), followed by *S. aureus* (22.9%), while *Klebsiella pneumoniae* (*K. pneumoniae*) represented 11.4% of the isolates as shown in Table 2.

The results of the 35 pregnant women with ASB showed that their median age, and BMI was 27.8 ± 5.5 years, and 25.49 ± 6.61, respectively. Of those subjects, 12 (34.3%) were in their 1st trimester, 11 (31.4%) in the 2nd trimester, and 13 (37.1%) in the 3rd trimester. The results of the dip-stick urine analysis showed that 31 (88.6%) had normal urobilinogen, 27 (77.2%) had negative protein, 33 (94.4%) had negative ketones, 20 (77.1%) had positive leukocyte esterase, 18 (51.4%) were positive for nitrites, and only 20.0% showed traces of blood. The microscopic urine analysis showed that 14 (40.0%) of the positive cases had pus cells > 5/HPF, 13 (37.1%) had RBC > 5/HPF, 30 (85.7%) had increased epithelial cells, and 34 (97.2%) had bacteria in their urine.

Table 2. Isolated organisms cultured from the positive urine samples (first and second samples). Data shown are frequencies (n and %).

Uropathogen	n (%)
E. coli	16 (45.7)
S. aureus	8 (22.9)
K. pneumoniae	4 (11.4)
E. faecalis	2 (5.7)
S. agalactiae	2 (5.7)
S. epidermidis	2 (5.7)
S. hominis	1 (2.9)
Total	35 (100)

E. coli: *Escherichia coli*, *E. faecalis*: *Enterococcus faecalis*, *K. pneumoniae*: *Klebsiella pneumoniae*, *S. aureus*: *Staphylococcus aureus*, *S. epidermidis*: *Staphylococcus epidermidis*, *S. hominis*: *Staphylococcus hominis*, *S. agalactiae*: *Streptococcus agalactiae*.

Regarding the antibiotic sensitivity profiles of the isolates; while most of the isolated Gram-positive organisms were sensitive to many of the tested antibiotics (Table 3), most of the detected Gram-negative isolates were resistant. *E. coli* isolates were resistant to ampicillin, sulfamethoxazole/trimethoprim, cefepime, cefotaxime, ceftazidime, amoxicillin, and nitrofurantoin at frequencies of 87.5%, 68.7%, 50.0%, 43.7%, 43.7%, 37.5%, and 6.3%, respectively. *K. pneumoniae* isolates were resistant to ampicillin, amoxicillin, sulfamethoxazole/trimethoprim, cefotaxime, cefepime, ceftazidime, and nitofurantoin at frequencies of 75.0%, 75.0%,75.0%, 50.0%, 50.0%, 25.0%, and 25.0%, respectively.

Table 3. Antibiotic sensitivity profiles of the isolated Gram-positive uropathogens from women investigated for asymptomatic bacteriuria. Data shown are frequencies; n (%).

Organism		Linz		Ery		Van		Clin		Nit		SXT		Amp	
		S	R	S	R	S	R	S	R	S	R	S	R	S	R
S. aureus (n = 8)	n	7	1	5	3	8	0	5	3	8	0	7	1	8	0
	%	87.5	12.5	62.5	37.5	100	0	62.5	37.5	100	0	87.5	12.5	100	0
E. faecalis (n = 2)	n	2	0	0	2	2	0	1	1	2	0	2	0	1	1
	%	100	0	0	100	100	0	50	50	100	0	100	0	50	50
S. agalactiae (n = 2)	n	2	0	2	0	2	0	1	1	2	0	2	0	2	0
	%	100	0	100	0	100	0	50	50	100	0	100	0	100	0
S. epidermidis (n = 2)	n	2	0	0	2	2	0	1	1	2	0	2	0	1	1
	%	100	0	0	100	100	0	50	50	100	0	100	0	50	50
S. hominis (n = 1)	n	1	0	1	0	1	0	1	0	1	0	1	0	1	0
	%	100	0	100	0	100	0	100	0	100	0	100	0	100	0

Amp: ampicillin, Clin: clinamycin, *E. faecalis*: *Enterococcus faecalis*, Ery: erythromycin, Linz: linezolid, Nit: nitofurantoin, *S. aureus*: *Staphylococcus aureus*, *S. epidermidis*: *Staphylococcus epidermidis*, *S. hominis*: *Staphylococcus hominis*, *S. agalactiae*: *Streptococcus agalactiae*. SXT: sulfamethoxazole/trimethoprim. Van: vancomycin. S: sensitive, R: resistant.

Discussion

Only very few studies have been conducted in Saudi Arabia to evaluate the prevalence of ASB among pregnant women and the studies showed heterogeneity in the results with a prevalence rate ranging from 1.7% to 23.34% [3,11]. This necessitated conducting the current study to assess the current extent of this problem, particularly in the northern part of the country where no previous investigations have been carried out.

In this study, the prevalence of ASB was 8.25% (35/400) and this agrees with the 7.8% prevalence rate reported among 102 pregnant women attending selected health facilities in Cameroon [4]. This is also in accordance with the prevalence rate of 7.8% reported in a study on Egyptian pregnant women [12]. A 10.0% rate was reported in two Egyptian tertiary hospitals [13]. Similarly, a 10.5% rate was reported among Indonesian pregnant women [14].

A lower prevalence rate (3.76%) of ASB was demonstrated in a cross-sectional study that included 587 pregnant women attending Mbale Hospital, Eastern Uganda [15]. Lee and his colleagues reported a prevalence rate of 4.5% in their community-based cohort study in Sylhet district, Bangladesh performed on 4242 pregnant women (< 20 weeks gestation) [16]. Nguefack and his team reported a prevalence rate of 5.7 in their cross-sectional study conducted in three hospitals in Douala that included 354 pregnant women [17].

On the other hand, higher rates of ASB were reported in other geographical areas. For instance, a 13.0% prevalence rate was detected among Iranian pregnant women [18]. In a study conducted at the Khartoum North Teaching Hospital a rate of 14.7% was reported [19]. Edae and his team [20], and Tadesse and his colleagues [21] reported higher rates of 19% and 21.2% among pregnant women in Eastern and Northern Ethiopia, respectively. Likewise, in Nairobi Kenya, a 21.5% prevalence rate was also reported [22]. A higher rate of 33.3% was reported among pregnant women compared to 7.0% among non-pregnant women in Ghana [2].

As stated previously the differences in sample size, geographical location, socio-economic status, and educational level, setting of study (primary care, general hospital, and community), variation of screening tests applied, and the cut-off point for the detection of pathogens, may all contribute to these vast differences in prevalence data.

In the current investigation non-significant correlations ($p > 0.05$) were detected between positive urine culture results, age, and BMI, and this is in accordance with Nguefack and his colleagues who concluded that age and obesity had no statistically significant influence on bacteriuria [17]. On the contrary, it was reported that the age had a significant correlation with ASB ($p = 0.0005$) and most of the significant growth occurred in the 35-45 years age group [3]. Likewise, a significant association ($p < 0.001$) was found between age and the prevalence of ASB, and non-significant association ($p = 0.20$) was detected regarding the trimester [23].

Additionally, no significant correlations were detected between urine culture findings and pregnancy duration, parity, or gravidity in our study. This agrees with the report where gestational age and parity did not have any statistically significant influence on bacteriuria [17]. Contrary to the researchers who reported that both gravidity/parity have had a significant correlation ($p = 0.0005$) with ASB and most of the significant growth was observed in the multiparous, followed by grand multiparous [24]. Mahmoud and his colleagues reported that the incidence of UTIs in pregnant women was the highest in the multigravidas [25]. It was reported that gravidity was a strong risk factor associated with ASB where 55 (73.3 %) were third gravida or more [26]. Similarly, Tadesse and his team demonstrated that ASB was substantially correlated with women's age, wages, and gestational time [21], while Bahavana and his colleagues observed that 71.8% of the ASB cases were recorded within the third trimester [27].

In our study, significant positive correlations ($p < 0.05$) were detected between urine culture results and positive RBS, urine leucocytes esterase, urine nitrites, urine pus cells, urine RBCs, urine epithelial cells, and urine mucus. These agree with the results of Etminan-Bakhsh and his colleagues [28].

The most commonly prevalent uropathogens detected in our study were *E. coli* with a prevalence rate of 45.7% (16/35), followed by *S. aureus* isolates with a 22.9% (8/35) prevalence rate. These observations are comparable with the findings reported by many researchers [16,17,29,30] who demonstrated that the predominant microorganisms were also *E. coli* at rates of 42.4 %, 48.6 %, 47.5 %, and 42.5 %, respectively.

Other studies have shown higher predominance rates for *E. coli* as the most common organism, at 88.0%, 70.0%, and 60.1%, respectively [3,31,32]. Contrary to these observations, it was reported that *S. aureus*, *E. coli*, and coagulase-negative *Staphylococci* were the most common bacterial isolates from pregnant women's urine samples at Dessie Referral Hospital, Northeast Ethiopia [33]. *K. pneumoniae* was the third

most common prevalent pathogen in this study (11.2%), and this is comparable to the 12.5% prevalence rate reported by Lee and his colleagues [16], and the 14.3% rate reported by Nguefack and his research team [17].

In the current study, while most of the isolated Gram-negative organisms were resistant to many of the tested antibiotics, most of the detected Gram-positive isolates were sensitive. Hamden and his research team found that *E. coli* was resistant to a variety of antibiotics, most notably nitrofurantoin, amoxicillin, and co-trimoxazole [19]. On the contrary, it was reported that 79.5%, 50.0%, 47.7%, and 36.4% of the *E. coli* isolates were sensitive to nitrofurantoin, ceftazidime, cefotaxime, and co-trimoxazole, respectively [24]. Quadri and his colleagues found that most of the *K. pneumoniae* isolates were sensitive to cefotaxime, ceftazidime, and nitrofurantoin [34]. A comparable finding was reported by Ali and his research team who found that the bulk of the Gram-negative bacterial isolates was susceptible to nitrofurantoin, norfloxacin, ciprofloxacin, ceftriaxone, and amikacin at rates of 95.2%, 85.7%, 80.95%, 80.95%, 76.2%, respectively [33]. Similarly, it was found that all the Gram-positive isolates were sensitive to ampicillin, piperacillin-tazobactam, vancomycin, linezolid, cefepime, teicoplanin, and nitrofurantoin, but they were resistant to co-trimoxazole [35]. On the other hand, it was reported that 75.0% of the Group B *Streptococc*us isolates were sensitive to sulfamethoxazole/trimethoprim [34].

Thus, there were some similarities and differences in the rates of resistance towards the antimicrobial agents which could be explained by the fact that different roles and guidelines are practiced in different countries and there is wider abuse of these drugs in the different communities which in turn influence the spread of resistance among the different uropathogens.

Conclusions

To the best of our knowledge this is the first study to evaluate the prevalence of ASB among pregnant females, identify the causative organisms and determine their antibiotic susceptibility patterns in the Maternity and Children's hospital, Arar, Saudi Arabia. The prevalence rate of ASB (8.75%) was within the general prevalence rate reported in many studies in Saudi Arabia. While most of the isolated Gram-positive organisms were sensitive to many of the tested antibiotics, most of the detected Gram-negative isolates were resistant. Although the Ministry of Health in Saudi Arabia provides comprehensive antenatal care for all pregnant women with all the required investigations, it does not include urine culture for diagnosis of ASB. ASB caused by antibiotic resistant organisms is alarming. Screening for ASB during pregnancy using urine culture and sensitivity testing is of vital importance to improve the maternal and neonatal outcome.

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Author contribution

Conceptualization, Amjad Alenzi, Ibrahim Taher, Amany Ghazy, Waleed Elawamy and Ahmed Taha; Data curation, Ibrahim Taher, Amany Ghazy, Waleed Elawamy, Ahmed Alshash, Eman El-Masry and Ahmed Taha; Formal analysis, Ibrahim Taher, Amany Ghazy, Waleed Elawamy and Ahmed Taha; Investigation, Ibrahim Taher, Amany Ghazy, Waleed Elawamy, Ahmed Alshash, Eman El-Masry and Ahmed Taha; Methodology, Amjad Alenzi, Ibrahim Taher, Amany Ghazy, Waleed Elawamy, Eman El-Masry and Ahmed Taha; Project administration, Ibrahim Taher, Amany Ghazy, Waleed Elawamy, Ahmed Alshash and Ahmed Taha; Resources, Amjad Alenzi, Ibrahim Taher, Amany Ghazy, Waleed Elawamy and Ahmed Taha; Software, Ibrahim Taher, Amany Ghazy, Waleed Elawamy and Ahmed Taha; Supervision, Ibrahim Taher, Amany Ghazy, Waleed Elawamy, Ahmed Alshash, Eman El-Masry and Ahmed Taha; Validation, Ibrahim Taher, Amany Ghazy, Waleed Elawamy, Ahmed Alshash and Ahmed Taha; Visualization, Ibrahim Taher, Amany Ghazy, Waleed Elawamy and Ahmed Taha; Writing – original draft, Amjad Alenzi, Ibrahim Taher, Amany Ghazy, Waleed Elawamy, Ahmed Alshash, Eman El-Masry and Ahmed Taha; Writing – review & editing, Ibrahim Taher, Amany Ghazy, Waleed Elawamy, Eman El-Masry and Ahmed Taha. All the authors have read and agreed to publish the manuscript in its current form.

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