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Antimicrobial resistance patterns in acquired urinary and genital tract infections

Abstract

Objective: The aim of this study was to demonstrate the efficacy of antibiotics on isolates from urinary and genital tracts of Jordanian women where better understanding of antibiotic treatment policy may lay out.

Method: This study was conducted during summer 2016 where total of 268 patients were referred to the laboratory for urine and HVS culture out of which 132 revealed microbial growths. One hundred and ninety seven (197) midstream urine samples were collected in the laboratory while seventy one high vaginal swabs were referred. Samples were inoculated in duplicate aseptically and incubated aerobically at 37°C. Colony forming units were counted 24 hours later, and if count ≥ 100,000/ml, antimicrobial sensitivity studies were then performed and read after a further 24 hours. Nutrient, blood, Chocolate and MacConkey agar media were used for HVS. Plates were inoculated in duplicate aseptically and were incubated under aerobic and anaerobic condition at 37°C for 24 hours. Microorganism identification and characterization using routine morphological and biochemical methods were applied. Mueller Hinton Agar (MHA) was the selected medium for sensitivity test. Commercial multidisc and single sensitivity discs were used for susceptibility test. Zone of inhibition was then measured to determine the level of susceptibility of isolates to antibiotics. Data obtained in this study were analyzed using SPSS version16.0

Results: One hundred and one (51.3%) and 31 (43.7%) revealed positive growth for urine and HVS respectively. The rate of urinary tract Infection (UTI) was significantly higher than genital tract infection (P<0.001). *Escherichia coli* revealed the highest prevalence 38 (37.6%) in urinary tract, while *Staphylococcus aureus* was the highest isolates in genital tract 8(25.8%). *S. aureus* and coliforms showed the next highest rates 16(15.8%) and 15(14.9%) respectively followed by *Klebsiella pneumonia* 14(13.9%) for urine samples. Other bacterial species include *Staphylococcus epidermidis*, *Streptococcus feacialis*, *Proteus mirabilis* and *Bateriodes* were isolated with various prevalence rate. The prevalence of *Candida albicans* for HVS and urine was 10(32.3%) and 6(5.9%) respectively while *Lactobacillus spp.* was solely isolated from HVS. Meropenem and imipenim showed 100.0% for urine isolates followed by levoloxacin 97.9%, 99.3%.They were the most active antibiotics, followed by tobramycin (95.5%,92.2%), cefoxitin (89.5%,94.1%) and ciprofloxacin (89.5%,86.2%). No statistical significant variation in drug susceptibility between isolate from urine and HVS. The percentage of multidrug resistance was 61.1% and (57.1%) for urine and HVS isolates respectively. The rate of multidrug resistance was higher than 50.0% for both urine and HVS samples.

Conclusion: The high resistant rate of to antibiotics should be considered. There was no statistical significant variation in drug susceptibility between isolate from urine and HVS .The rate of multidrug resistance in both samples is high (>50%).These results are worrisome and essential care should be taken in maternity unit and drug prescribing policy should be monitored and updated.

Keywords: efficacy, urinary tract, hvs, multidrug resistance

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Abbreviations: UTI, Urinary Tract Infection; MHA, Mueller Hinton Agar; MDR, Multidrug Resistant Bacteria; AMX, Amoxicillin; AUG, Augmentin; FOX, Cefoxitin; CRO, Ceftriazone; COT, Cotrimoxazole; CPX, Ciprofloxacin; GEN, Gentamycin; IPM, Imipenim; LEV, Levofloxacine; NA, Nalidixic Acid; NIT, Nitrofurantoin; NOR, Norofloxacin; PFL, Pefloxacin; TOB, Tobromycin

Introduction

The complication caused by urigenital infection and specifically by multidrug resistant microbes can cause serious problems for women especially during pregnancy although Pregnancy doesn't cause urinary tract infection (UTI) however; the physical changes occur during pregnancy increase susceptibility of pregnant women to infection.[1–3] Women in general tend to visit hospitals and gynecology clinic for routine checkup regardless of sign of infections.[4–6] Urinary tract infection, cystitis, vaginitis and pyelonephritis are the most common cause of uriginital infections.[4,7] The incidence of asymptomatic infections were increased by hypertension, still birth, abortion, preterm labor and thrombosis.[3,6,8] Significant maternal and fetal morbidity and mortality occur due to pyelonephritis.[3,9] Hormonal changes during women cycle provide the ideal environment for UTI-causing bacteria (*Escherichia coli*) and increase rate of infection, like wise women vagina work as niche for bacterial growth and *Candida* due to moisture habitat and rich glycogen content.[10] Although most of the vaginal bacteria are not pathogenic unless they have chance to overgrown in numbers or in case of an abrasion they will induce infection. Due to these facts women are more susceptible to

infection which even increased during pregnancy.[1–3] Exposure of women during routine visit to hospital and antenatal clinich where nosocomial infection rate is high will increase chance of infection. This contact has been reported to cause serious problem in developing countries where drug prescribing policy is not controlled and random antibiotic treatment developed multidrug resistant bacteria (MDR) making infection treatment difficult task and therefore will enhance threat to women and fetus in case of pregnancy and will reduce rate of using safe antibiotic and hence treatment successes hard to achieve and infection impossible to eliminate.[4,11–13] Many studies focused on (MDR) bacteria developed in hospital and clinic especially methicillin resistant *Staphylococcus aureus* which create one of the major serious problems in treating patients exposed to such environment. The demographic situation plays an important role in reporting gram negative and gram positive MDR showing increase rate of gram negative MDR where gram positive MDR suppose to be dominant. These finding illustrate an urgent indication for a better drug surveillance control system.[4,14] In both cases MDR rate is high leading to possible formation of modified MDR strains by either intrinsic manner or by mutation.[14,15] specifically in underdeveloped countries where prescribing antibiotics is not controlled therefore better surveillance drug policy is required.[7,16] Most studies focused on prevalence of UTI with less concern of genital tract infection.[3] The scope of this study is to lay out the prevalence of microbes, identity and drug resistant pattern for isolates from both urinary and genital tracts in Jordanian women.

Material and methods

A total of 268 patients were referred to the laboratories as outpatients attending antenatal clinics for routine examination. Suspecting patients with UTI were referred for further investigations. Clean-catch MSU samples were collected in wide open mouth sterile container as described by.[17,18] Patients were instructed to wash and clean urethro-genital organs and to pass the first void of urine then to collect midstream sample into sterile container. Samples were labeled and analyzed within 30 minutes of collections. Urine samples were examined microscopically according to.[19] Those showed 10 white blood cells/ mm3 were regarded as pyuric.[20] Urine culture was then performed. Identification and characterization of isolated bacteria include Gram stain followed by microscopic examination, motility test and biochemical tests as described by.[21–24] For drug susceptibility test Mueller Hinton agar (MHA) was used and commercial antibiotic multidisc were used as described by.[23] Antibiotics discs, Antibiotics discs; amoxicillin (AMX) 25/mlμg, augmentin® (AUG) 30μg/ml, cefoxitin (FOX) 30μg/ml ceftriazone (CRO) 30μg/ml, cotrimoxazole (COT)ug/ml, ciprofloxacin (CPX) 10μg/ml, gentamycin (GEN) 10μg/ml, imipenim (IPM)10μg/ml, levofloxacine (LEV) 30μg/ml, nalidixic acid (NA) 30μg/ml, nitrofurantoin (NIT) 20μg/ml, norofloxacin (NOR) 30μg/ml, pefloxacin (PFL) 5μg/ml, tobromycin (TOB) 10μg/ml were used. Zone of inhibition was measured to determine the level of susceptibility of isolates to the antibiotics. Data obtained in this study were analyzed using SPSS version16.0.[23] Samples were inoculated in duplicate aseptically and incubated aerobically at 37°C and an aerobically for HVS. Colony forming units were counted 24 hours later, and c if ≥100,000/ml, antimicrobial sensitivity studies were then performed and read after a further 24 hours.

Results

A total of 268 patients were referred to the laboratory for urine and HVS culture out of which 132 revealed microbial growth giving prevalence rate of 101(51.3%) and 31(43.7%) for urine and HVS sample respectively. The rate of UTI was significantly higher than genital tract infection ($P< 0.05$). The prevalence and the rate of positive culture revealed single isolate was (74.6%) and (66.3%) for urine and HVS respectively however, the number and rate of samples showed mixed growth culture was 24.6% and (21.4%) for urine and HVS samples respectively. *E.coli* was the most predominant organism with prevalence rate of 38(37.6%) in urine samples while *S.aureus* showed the highest prevalence rate 8(25.8)% for HVS followed by *C. albicans* 10(32.3%). Summary of the results presented in Table 1.

Table 1 Prevalence of microorganisms isolated from Urine and HVS samples

Organism	Urine		HVS		Total	
	Freq. of Occurrence	% Prevalence	Freq. of Occurrence	% Prevalence	Freq. of Occurrence	% Prevalence
E.coli	38	37.6	4	12.9	42	31.8
S. aureus	16	15.8	8	25.8	24	18.2
Coliforms	15	14.9	2	6.5	17	12.9
K. pneumonia	14	13.9	0	0	14	10.6
S. epidermis	4	4	1	3.2	5	3.8
S. faecialis	3	3	1	3.2	4	3
Bacteriodes	2	2	0	0	2	1.5
P. mirabilis	1	0.9	0	0	1	0.8
P. aeuroginosa	2	2	1	3.2	3	2.3
Lactobacillus	0	0	4	12.9	4	3
C. albican	6	5.9	10	32.3	16	12.1
Total	101	100	31	100	132	100

Meropenem and norfloxacin showed 100.0% and 99.3% activity for urine and HVS isolates respectively followed by ipipenim 100.0%, 97.4%, levoloxacin 98.9%, 99.3%, They were the most active antibiotics followed by tobramycin 95.5%, 92.2%,Cefoxitin 89.5%, 94.1% and ciprofloxacin 89.5%, 86.2%. There was no statistical significant variation in drug susceptibility between isolate from urine and HVS. In this study multidrug resistance (MDR) is described as the capacity of isolate to resist minimum concentration of certain drug. The rate of MDR in urine sample was higher than HVS sample 61.1%, 57.1% respectively Table 4 and 5 however, there was no statistical significant difference noticed, this indicate that UTI was more expose to drug treatment than genital tract infection. The rate of multidrug resistance in both samples is high (>50%). These results are worrisome and essential care should be taken in pregnancy unit and efficient drug prescribing policy should be explored.

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Discussion and conclusion

The incident UTI showed statistical significant ($p<0.05$) over HVS which might be related to site of collection however, *Candida albicans* (*C.albicans*) showed higher incident rate in samples isolated from HVS 10(32.3 %) than urine 6(5.9) because female genital tract has suitable condition supporting growth of different types of aerobic and anaerobic bacteria as well as *C. albicans*.[23] Result of this study indicate the presence of mixed culture as well as MDR organisms which agree with other study reported.[24] where antibiotic selection treatment policy is difficult, especially in the developing countries, where inadequate health services strategy, uncontrolled drug prescriptions surveillance, and improper treatment strategies all lead for development of increase incident rate of microbial resistance and developing of MDR.

The overall prevalence (51.3%) and 31(43.7%) for urine and HVS respectively, found in this study are rather high however. The HVS prevalence rate is not as high as but rather close to the (40.1%) study reported by .[4] however, it is inconsistent with other study showing prevalence rate of 12.7%.[23] This is quite understandable due to different geographical location and social and economical status of women. *E.coli* was the most prevalent organism followed by *S.aureus* in urine samples however; *S.aureus* was the most prevalent bacterial isolate from genital tract followed by *E. coli* Table1. This result agree with other studies.[4,9] Although organisms isolated in this study showed lower prevalence in uriginital infection which is consistent with other studies.[17,23] however, their prevalence and resistant pattern should be taken in consideration. *E.coli*, *K. pneumonia* and *Ent. faecalis* also showed a resistance pattern >50% for most of drug used as first line of treatment. This is consistent with other studies.[4,14] however, it is inconsistent with the sensitivity rates (75% and 74%) for *E. coli* and *K. pneumonia* respectively, to antibiotics reported by Momoh ARM et al.[14] & Akerele et al.[3]

Ciprofloxacin showed activity rate of 89.5% and 86.2%, nalidixic acid showed activity rate 88.4% and 85.0% for urine and HVS respectively. Other antibiotics explicit high activity, ofloxacin 87.7% and 82.4%, pefloxacin 80.2% and 72.5% for isolates from the respective samples without showing significant statistical variation. The results of antibiotics activity agreed with other studies.[4,14] The high activity of flouroquinolones in this study is rather interesting for both urine and HVS samples Table 2 and 3. It is not surprising due to the unique action of the drug on DNA inhibition and as documented flouroquinolones are newer drugs with mode of action central on inhibition of the DNA.[14,23–26] Adding to that flouroquinolones groups are relatively expensive therefore patients exposure to the drug is low.[4]

Table 2 Susceptibility of bacteria isolated from midstream urine sample to various antibiotics

Bacteria	*E.coli*	*S.aureus*	*Coliforms*	*K.pneumonia*	*S.epidermis*	*S.faecialis*	*Bacteriode*	*P.mirabilis*	*P.aeuroginosa*	T
No / % susceptibility	38	16	15	14	4	3	2	1	2	95
AUG 30 ug	18 47.4	7 43.7	11 73.3	6 42.8	3 75	2 66	1 50	0 0	1 50	49 51.6
CRO 30 ug	16 42.1	6 37.5	12 80	8 57.1	2 50	2 66	2 100	0 0.0.	2 100.0.	50
NIT20 ug	19 50	11 58.8	11 73.3	9 64.2	2 50	2 66	1 50	0 0	0 0	55 57.9
GEN 10 ug	12 31,6	4 25	11 73.3	6 42.8	4 75	2 66	1 50	0 0	1 50	41 43.1
COT 25	15 39.5	13 81.3	14 93.2	14 100	4 75	2 66	1 50	0 0	2 100	65 68.4
OFL 5 ug	26 68.4	14 87.5	16 100	8 78	4 75	2 66	2 100	0 0	1 50	73 76.8
AMX26	2 5.2	7 43.7	8 53.3	2 14.3	2 50.0	1 33	1 50	0 0	0 0	23 24.2
CIP 5 ug	38 100	15 93.8	15 100	13 86.7	4 100	3 100	2 100	1 100	2 100	93 97.8
PF5 ug	24 63.2	14 87.5	14 93.2	13 86.7	3 75	2 66	1 50	0 0	1 50	72 68
NA30 ug	29 76.3	15 93.8	13 86.7	14 100	4 100	2 66	2 100	1 100	1 50	81 85.3
LEV10 ug	38 100	15 93.8	15 100	14 100	4 100	2 66	2 100	1 100	2 100	93 97.9
IPM30	38 100	16 100	15 100	14 100	4 100	3 100	2 100	1 100	2 100	95 100
NOR 10 ug	35 92.3	13 81.3	15 100	13 86.7	3 75	2 66.6	1 50	1 100	2 100	85 89.5
FOX30 ug	34 89.5	13 81.3	14 93.3	13 86.7	3 75	2 66.6	2 100	1 100	1 50	83 87.3
MEM 10ug	38 100	16 100	15 100	14 100	4 100	3 100	2 100	1 100	2 100	95 100
TOB10 ug	38 100	14 87.5	15 100	13 86.7	3 75	2 66.6	1 50	1 100	1 50	88 92.6

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Table 3 Susceptibility of bacteria isolated from HVS to various antibiotics

Bacteria	*E.coli*	*S.aureus*	*Coliforms*	*K.pneumonia*	*S.epidermis*	*S.faecialis*	*Bacteriode*	*P.mirabilis*	*P.aeuroginosa*	Total
No / % susceptibility	4	8	2	0	1	1	0	0	1	17
AUG 30ug	1 25	3 37.5	1 33.3	0 0	1 50	0 0	0 0	0 0	0 0	6 35.3
CRO 30ug	2 50	3 33.5	1 50	0 0	1 100	0 0	0 0	0 0	1 100	8 47.1
NIT20ug	3 75	5 62.5	1 50	0 0	0 0	0 0	0 0	0 0	0 0	9 52.9
GEN 10ug	2 50	4 50	1 50	0 0	0 0	0 0	0 0	0 0	0 0	7 38.9
COT 25	2 50	4 50	1 50	0 0	1 100	0 0	0 0	0 0	1 100	8 47.1
OFL 5ug	2 50	7 87.5	1 50	0 0	0 0	0 0	0 0	0 0	1 100	11 64.7
AMX26	0 0	3 37.5	1 50	0 0	0 0	0 0	0 0	0 0	0 0	4 23.9
CIP 5ug	3 75	5 62.5	1 50	0 0	0 0	1 0	0 0	0 0	1 100	11 64.7
PF5 ug	3 75	6 75	1 50	0 0	0 0	1 100	0 0	0 0	0 0	11 64.7
NA30 ug	3 75	7 87.5	1 50	0 0	1 100	1 100	0 0	0 0	0 0	13 76.5
LEV10 ug	4 100	8 100	2 100	0 0	1 100	1 100	0 0	0 0	1 100	17 100
IPM30	4 100	8 100	2 100	0 0	1 100	1 100	0 0	0 0	1 100	17 100
NOR 1o ug	4 100	7 87	2 100	0 0	1 100	1 100	0 0	0 0	1 100	16 94.4
FOX30 ug	4 100	8 100	2 100	0 0	0 0	1 100	0 0.0	0 0	0 0	15 88.2
MEM 10 ug	4 100	8 100	2 100	0 0	1 100	1 100	0 0	0 0	1 100	17 100
TOB10 ug	4 100	7 87.9	3 100	2 100	0 0	0 0	0 0	0 0	0 0	30 94.1

Nitrofurantoin, augmentin® ceftriazone and gentamicin showed moderate effective range on urine and HVS isolates 45.0% to 55.0%. There was no significant statistical variation in drugs susceptibility of urine and HVS isolates to augmentin® and ceftriazone, which is in agreement with other study.[4] however, nitrofurantoin showed 57.3% and 54.9% to urine and HVS respectively with no significant statistical variation. This result is slightly different from other study.[4] reported higher activity for urine isolates over vaginal isolates. This might be due to geographical difference between countries and their antibiotics prescription policy.

Activity of gentamicin 47.1% and 44.7% for vaginal and urine sample respectively with no statistical significant variation however, it is in agreement with other study where effect of gentamycin in treating vaginal infection was higher than UTI.[4] This indicate rational use of gentamicin in genital infection treatment in Jordanian pregnant women as first line of treatment for the fact that gentamicin is safe to treat pregnant women.[27] Amoxicillin showed very low efficacy (<30%) this is not surprising result since amoxicillin was used widely for treatment of various type of infection caused formation of drug resistant strains. This result is in agreement with other studies showed high resistance microbes to amoxicillin.[7,21] Therefore, it is not recommended to use amoxacillin for UTI and vaginal infection.

The results obtained in this study show clearly the disadvantages of inappropriate use of antimicrobial agents obviously minimize options of using selective drugs for future uriginital infection treatment. The high resistant of urine isolates and the formation of MDR strains can be explained by temovedhe fact that the urine isolates were exposed to consistently higher urine concentrations of antimicrobial agents, compared to low antibiotic concentration of HVS. Urine samples also showed higher rate of multidrug resistance than HVS isolates 61.1% and 57.1 respectively, with significant statistical variation ($P<0.05$) due to different location of isolates.[1–3]

There was no specific pattern of drug resistance for urine and HVS isolates however, *S.aureus* in both specimen interestingly showed the highest multidrug resistance of 73.6% and 63.5% (Mean = 68.6%), followed by *P.auregonosa* 75.0% and 66.6% (Mean=70.8%). *E. coli* showed 67.0% and 60.6% (Mean=63.8) resistance rate. The high resistance of *S.aureus* is not surprising because the bacterium is documented for exhibit high resistance rate.[3,5,6] since it is a commonly found in hospital environment, antenatal clinics and community.[28] The prevalence of multidrug resistant *S.aureus* in this study demonstrate a resistance profile of >50% to almost half of the antibiotics tested, part of these selected antibiotic usually used as first line treatment. The resistance scheme of *S.aureus* is consistent with other studies.[14,29] where *S.aureus* resistant profile in clinics and hospital was documented and prevalence of methicillin resistance *S.aureus* strains were stated.[14,29,30] The resistant of *P.*

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aeruginosa to antibiotics is well documented, our results showed low effect of antibiotics on *P.aeruginosa* except for few selective group, flouroquinolones showed high activity rate which agree with other studies.[14,31] However, recommendation for surveillance on prescribing such antibiotic is highly recommend.

The result of this study showed low prevalence rate for *E.coli and E. faecalis*al though, these two microbes showed >60.0% and >50% resistant pattern respectively which agree with other study.[4] but disagreed to sensitivity rate of 75% and 74% reported by.[3] This can be explained by the fact that theses isolates have been exposed to various antibiotic concentrations in hospital for period of time leading to acquisition of antibiotic resistance by different mechanisms. Immense concern is recommended for treatment of future infection caused by these two organisms. Lactobacillus susceptibility to antibiotics was not of our interest in this study due to normal presence of this microorganism in women genital tract.

Generally, the level of multi-drug resistance illustrated in this study Table 4&5 is of great concern for women due to the fact that these antibiotics have less effect on isolates which will create serious problem in health profession strategy and requirement to reduce rational use of antibiotics is of priority concern, for further extension of antibiotics life spam.[32] Drug resistant in this study will add further problem for women treatment as already mentioned that high percentage of infection to treat women with asymptomatic and these symptoms will change to symptomatic infections if they did not diagnose early leading to infant morbidity and mortality in case of pregnancy.[33] therefore, the remarkable resistant of these isolates in this study is of great concern for laying out the base line for future policy of treatment and to avoid invention of new MDR strains.

Table 4 Prevalence of MDR Bacteria Isolates in Urine Samples

Isolated Bacteria	No Resistant to				Total MDR	% MDR
	No. of isolate	3 Drugs	4 Drugs	>5 Drugs		
E.coli	38	14	9	3	26	68.4
S.aureus	16	6	3	2	12	75
Coliforms	15	5	2	1	8	53.3
K.pneumonia	14	4	2	1	7	50
S.epidermis	4	1	0	0	1	25
S.faecialis	3	1	1	0	2	66.6
Bacteriodes	2	1	0	0	1	50
P.mirabilis	1	1	0	0	0	100
P.aeuroginosa	2	1	1		2	100
Total	95	34(38.2)%	18(18.9%)	7(7.3%)	59	62.1

Table 5 Prevalence of MDR Bacteria Isolates in HVS Samples

Isolated Bacteria	No Resistant to				Total MDR	% MDR
	No. of isolate	3 Drugs	4 Drugs	>5 Drugs		
E.coli	4	2	1	0	3	75
S.aureus	8	2	1	1	5	62.5
Coliforms	2	1	0	0	1	50
S.epidermis	6	1	0	0	1	16.7
S.faecialis	1	0	1	0	1	100
P.aeuroginosa	1	0	1	0	1	100
Total	22	6 (27.2%)	4 (18.2%)	1 (5.4%)	11	50

Isolate from urine and HVS samples showed high prevalence rate and high antibiotics resistance patterns although, some variation was noticed in their effective rate, such variation might be of concern for public health sector regarding urigenital infection and drug prescribing policy for women under treatment in particular and for prescribing antibiotics in general in countries where respected surveillance system absence. The results obtained in this study for rate of infection in urinary and genital tract and even for efficacy of antibiotics is in accordance with previous study by Battikhi M et al.[33] where he showed that isolates from urinary and genital tract of pregnant women showed similar rate, type of infection and antibiotic resistant.

Our recommendation is to carry out routine HVS and urine culture for all women to ensure good health for future pregnancy and fetus regardless sign of infections to provide back ground for further efficient control in antenatal care units and to provide better management policy in general and for pregnant women in particular. This will enhance reduction of the devastating effects of microbial infections and provide better health. The overall antimicrobial activity pattern showed that meropenem was the most effective antibiotic rate of 100.0% for urine and HSV isolates followed by Iepipenim, levoloxacin, norfloxacin, cefoxitin and tobromicin showed high activity rate Table 2 and 3. The above result gives strong indication for using these antibiotics for infections however, urgent policy for efficient surveillance system, drugs prescribing, treatment strategy and management in public health and even in the private sector should be implemented.

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Conflicts of interest

None.

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