Bacterial Causes of Renal Affection Associated With Pathological Changes in Buffalo Calves with Special Reference to Streptococcus Faecalis

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Abstract: Fifty urinary tract-infected cases were obtained from slaughtered buffalo calves aged 2-6 months at Cairo abattoirs during the period from May, 2012 to December, 2012. Specimens from the infected tissues (urinary bladders, ureters and kidneys) were subjected to bacteriological and pathological examinations. The bacteriological examination revealed that bacterial pathogens had been isolated including *Staphylococcus aureus* (22%), *Enterococcus faecalis* (10%), *Escherichia coli* (10%) and *Klebsiella pneumoniae* (2%) as single infection, while the mixed infections were (*E. faecalis+Staph*) (4%), (*E. faecalis+Klebsiella spp*.) (8%), (*Staph.+E.coli*) (6%), and (*E.coli+Coryne*) (2%) and the negative samples for bacteriological examinations were (36%). The virulence factors among the *Enterococcus faecalis* isolates were 37.5%, 50%, 12.5%, and 75.% for Haemolysin, Gelatinase, Aggregation factor and Biofilm formation, respectively. Antibiogram technique was applied for all isolates and it revealed that all the isolates were sensitive for Streptomycin, Amikacin, Gentamycin and Ofloxacin, while gram negative isolates were sensitive for Colistin and Ciprofloxacin, and all isolates were resist Ampicillin and Tetracycline. Meanwhile, the pathological findings could be classified the urinary affections into acute and chronic cystitis with focal ureteritis and nephrolithiasis. Acute cystitis was either hemorrhagic with intense aggregation of erythrocytes inside the lumen and lamina propria of urinary bladder, ulcerative and necrotic types. The chronic cystitis was follicular and suppurative types. The kidneys showed multiple stones with cystic dilation of the renal tubules and pyelonephritis.
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1.Introduction

Although the bladder and ureters are often exposed to bacteria and although urine generally supports bacterial growth, the combined effects of bladder emptying by urination and an intrinsic defense mechanism associated with bladder epithelium assist in resisting bacterial infection of the bladder and other urinary tract (**Cox and Hinman, 1961 and Nassan, 2009).**

Cystitis, or inflammation of the bladder, has a direct effect on bladder function. It can occur due to both infectious as well as noninfectious etiologies. Infections can be due to Gram negative microorganisms such as *Proteus*, *Klebsiella, Citrobacter, Enterobacter*, and *Pseudomonas* species and Gram-positive pathogens such as *Enterococcus faecalis, Staphylococcus saprophyticus,* and group B streptococci. However, *Escherichia coli* represent the most common cause of infectious cystitis **(Echols *et al.*, 1999).**

Pyelonephritis frequently occurs in cattle, buffaloes and to lesser extent sheep,it is a polybacterial infection that invariably include *Corynebacterium renale*, *C. pseudotuberculosis*, *Escherichia coli*,*Staphylococcus aureus*,*Streptococcus* spp.,*Enterococcus* spp.and *Klebsiella* spp**.(Van metre and Divera,2002).** Pathogenesis of pyelonephritis mainly depend on the abnormal reflux of bacterial contaminated urine from lower tract to the renal pelvis and collecting tubules **(Lucky,2003 and Rosenbaum *et al.*, 2005).**

Enterococci are apart of normal intestinal flora of humans and animals.They are gram positive non spore former organisms usually inhabiting the alimentary canals of humans and animals in addition to being isolated from environmental and animal sources, most infections are caused by *enterococcus faecalis* and *enterococcus faecium* (**Giridhara *et al.*, 2010**).Infections caused by genus enterococcus include urinary tract infections, bacteraemeia,intra-abdominal infections and endocarditis **(Mundy *et al.*, 2000).**

Hence a detailed study of the virulence factors can lead to a better understanding of the pathogenesis of enterococcal infections. Such virulence factors may play an important role in enhancing the pathogenicity and are expected to be associated with infections with a higher degree of severity as well as with nosocomial or hospital acquired infections. A number of studies have identified different virulence factors in enterococci. The most important among them are hemolysin, gelatinase, enterococcal surface protein [esp], aggregation substance [AS], serine protease,

capsule, cell wall polysaccharide and superoxide.**(Giridhara *et al.*, 2010).**

Staphylococcus aureus is frequently isolated from urine samples obtained from long-term care patients. The significance of staphylococcal bacteriuria is uncertain. We hypothesized that *S. aureus* is a urinary pathogen and that colonized urine could be a source of future staphylococcal infection**.(Robert et al,2006)**

E. coli is a normal inhabitant of lower intestine and abundant in faeces and environment **(Timonedy et al., 1988**). Uropathogenic *E. coli* cause 90% of the urinary tract infection. The bacteria colonize from the faeces or perineal region and ascend the urinary tract to the bladder. Uropathogenic *Escherichia coli* (UPEC) is a causative agent in the vast majority of urinary tract infections (UTIs), including cystitis and pyelonephritis, and infectious complications, which may result in acute renal failure in healthy individuals as well as in renal transplant patients. UPEC expresses a multitude of virulence factors to break the inertia of the mucosal barrier. (**Justyna *et al.*, 2012)**

C. renale type I has ability to produce kidney damage after 48 hrs. post inoculation revealed emboli glomeruler nephritis with less number of *C. renale*, also there is infiltration of polymorphnuclear inflammatory cell and nephrosis, in addition to vacuolar degeneration, coagulative necrosis with blood vessel congestion **(Saba, 2010).**

The aim of work: Is to isolate and identify the pathogens which cause the urinary tract infections,study the most important virulence factors of *Enterococcus feacalis*, application of antibiogram to know the drug of choice that affect on the isolated pathogens and study the pathological changes associated with the bacterial infections for the urinary tracts of buffalo calves.

2. Material and methods

Samples: the present study was carried out on (50) organ samples collected from apparently healthy slaughtered buffalo calves aged 2-6 months at the period from May 2012 to December 2012 included (20) ureters (20) urinary bladders and(10) kidneys obtained at Cairo abattoirs, each sample was taken under aseptic condition in a separate poly ethylene bag after being divided into two parts, one for the bacteriological exam. and the other kept in (10%) formalin for the pathological exam., and then transported directly to the laboratory for bacteriological and pathological examinations.

Bacteriological examination:

Under very aseptic conditions a small piece of each sample was cultured in nutrient broth tube then incubated at 37C for 24 hours, then an inoculum was cultivated on different selective media including 5% sheep blood agar, Mac Conckey agar, Mannitol salt agar, Edwards medium and brain heart agar,the plates were incubated aerobically at 37 c for 48 hours.The suspected colonies were examined for culture growth and morphology as well as biochemical tests according to **(Quinn *et al.*, 1994).**

Characterization and speciation of the isolates:

The isolates which were primarily identified as enterococcus were then further characterized to the species level with the help of conventional biochemical methods as devised by **(Facklam and Collins, 1989).**

Serological identification:was done by Lancefield test using Latex Reagent (Oxoid LTD, BASINGSTOKE, HANTS, ENGLAND - 2006).

Detection of virulence factors:

Haemolysin production: The ability of the enterococcal isolates to produce haemolysin was analysed by haemolysin assay. (**Roberta Creti *et al.*, 2004**).The isolates to be tested were plated on blood agar base supplemented with 5% human blood. The plates were incubated at $37C^0$ and observed after 24 hrs and 72 hrs. Plates were observed for alpha, beta and gamma hemolysis.

Gelatinase production:

Gelatinase production was detected qualitatively by inoculating the clinical isolates on to gelatin agar and incubating at $35C^0$ for 24 hrs as standard method**.(Whaley *et al.*, 1982**) The growth of the isolates on the plate was then flooded with Frazier solution. A clear zone around the colonies indicated the digestion of gelatin and production of gelatinase by the organism.

Production of aggregation substance (AS): Clumping assay:

The assay as described by (**Roberta Creti *et al.*, 2004)** was used Briefly, 200µL of an 18 hour old culture supernatant of the *E. faecalis* strain grown in Brain heart infusion broth, was added on to each of the enterococcal strains being tested. After incubation at $37C^0$ for 24 hrs, Presence or absence of bacterial clumping was directly visualized and reported.

Biofilm production:

The method of Jayanthi ***et al.*,** was followed. **(Jayanthi *et al.*, 2008**) Biofilm was detected by inoculating the isolates into trypticase-soya broth [TSB] with 0.5% glucose and incubating at 37ºC. After overnight incubation, the culture was diluted 1:40 in fresh TSB-0.5% glucose. 200µl of the diluted solution was added to flat-bottomed polystyrene microtiter wells and incubated for 48 hours at 37ºC. Wells were gently washed three times with distilled water. After drying the plates in an inverted position at room temperature for 1hour, the adherent biofilm was stained with 0.1% safranin and allowed to stand for 20 minutes at room temperature. Absorbance of

the biofilm on the bottom surface of each well of the dried plates was determined at 490 nm in an enzyme-linked immunosorbent assay (ELISA) reader. Test was carried out in triplicate and the average of the three optical density (OD) values was taken. Culture medium without organism was taken as blank. Biofilm producing *E. faecalis* strain was taken as positive control. Mean OD value of positive control was taken as standard. Those values above 0.2 were considered as high biofilm producers. Values below 0.081 were categorized into non-biofilm producers. OD values above the standard but within 0.081 and 0.2 were taken as moderate biofilm producers.

Antibiogram technique:

Antibiotic sensitivity test for all isolated bacteria was done using standard disc technique according to **(Boone and Castenholz, 2001)**.

Pathological Examination:

Collected samples were examined grossly for any gross lesions and then fixed in 10% buffered neutral formalin solution and processed routinely then five-micron thick paraffin sections were prepared, stained by H&E and then examined microscopically for histopathology (**Bancroft and Gamble 2008**).

3.Results

1-Results of bacteriological examination:

Table (1) shows the incidence of isolated pathogens from urinary tracts of buffalo calves, the percentages of the isolated pathogens from ureters were (25%,15%,10% and 0%) *for Staphylococcus aureus*,*Streptococcus faecalis, Escherichia coli* and *Klebsiella pneumoniae,* respectively, while (25%)of the ureter samples were negative for bacteriological examinations. In case of urinary bladder samples the percentages of isolated pathogens were (30%, 10%, 15%, and 5%), respectively, while (25%) of the samples were negative, the percentages of isolated pathogens from the kidneys were (20%) for *Staphylococcus aureus* and *Escherichia coli* as a mixed infection, meanwhile 80% of the samples were negative. The mixed infections were(*E.faecalis*+*Staph*)in10% of ureters,(*E.faecalis*+*Klebsiella* spp.)in5%of ureters and in15%of urinary b.,(*Saph.*+*E.coli)*in5%ofureters and 20%of the kidneys, and(*E.coli*+*Coryne*) in 5%of the ureters. Table (2) shows the prevalence of the virulence factors among the *Enterococcus faecalis* isolates which were (37.5%, 50%, 12.5%, and 75%) for Haemolysin production, Gelatinase production, Aggregation factor and Biofilm formation respectively.

Table (3) shows the Antibiogram of the isolated organisms from urinary tracts of buffalo calves, it was clear that all the isolates were sensitive for Streptomycin, Amikacin, Gentamycin and Ofloxacin, while gram negative isolates were sensitive for Colistin and Ciprofloxacin, and all isolates were resist Ampicillin and Tetracycline

2-Pathological results:

Pathological examination were carried out on the positive samples for bacteriological examination and described as

Acute cystitis:

Bacterial Isolates:

All single infections with *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli* and one of mixed infection of *Klebsiella spp* with *Enterococcus spp* were isolated from the acute cystitis cases.

Pathological Findings:

Macroscopically, severe congestion and hemorrhage were seen in the mucosa of urinary bladder with hematuria or flaxes of dark red clotted blood in the lumen. Sometimes the bladder showed marked thickening in the wall, focal ulceration of mucosa and congestion of serosa. Microscopically, acute hemorrhagic cystitis with massive erythrocytes aggregation inside the lumen and in the submucosa were noticed with *Enterococcus faecalis* besides necrotic mucosa, marked congested blood vessels and few leukocytes infiltrations (**Figs 1 and 2**). Papillary hyperplasia of the lining epithelium (**Fig 3**) with hydropic degeneration and few neutrophils infiltrations in the subepithelial zone (**Fig 4**) were observed with *Staphylococcus aureus*. Focal area of epithelial denudation with bacterial colonization and leukocytes infiltrations were also detected (**Fig 5**). Mild cystitis with severe congested submucosal blood vessels and edema (**Fig 6**) were noticed with *Escherichia coli*. The edema became prominent under focal necrotic mucosa (**Fig 7**). Some blood vessels were recently thrombosed and showed a mild perivascular lymphocytic aggregation. Focal ulcerations with adjacent hyperplastic mucosal epithelia containing Malakoplakia inclusions were visualized (**Fig 8**). Sloughing or denudation of the mucosa from the lining epithelium was seen in the mixed infection of *Klebsiella spp* with *Enterococcus spp*. Intense edema was seen in the lamina propria with few leukocytes infiltrations of mostly neutrophils (**Fig 9**). Focal replacements of the mucosa with mononuclears and few neutrophils were noticed (**Fig 10**). These cells were invaded to the lamina propria and submucosa (**Fig 11**) besides desquamation and necrosis in the lining epithelia. The ureters of infected cases were normal.

Chronic Cystitis:

Bacterial Isolates:

The mixed infections (*Escherichia coli* + *Corynebacterium spp* and *Enterococcus faecalis* +*Staphylococcus aureus*) were the isolates from the chronic cystitis cases.

Pathological Findings:

Macroscopically, the wall of the bladder was markedly thickened, gray, nodular, firm and with pale mucosa. Focal areas of congested, elevated and ulcerated mucosa were also noticed. The urine in these cases was cloudy and turbid. Microscopically, chronic cystitis with clumps proliferated submucosal lymphocytes forming follicular aggregations were seen just beneath the normal epithelial layer (**Fig 12**). The aforementioned finding was identified with mixed infection of *Escherichia Coli* with *Corynebacterium spp*. Multifocal abscesses in the submucosa (**Fig 13**) with huge numbers of neutrophils and round cells (**Fig 14**) were associated with mixed infection of *Enterococcus faecalis* and *Staphylococcus aureus*. Hyaline degenerated was noticed in the smooth muscles adjacent these abscesses (**Fig 15**). The mucosa was focally necrotic and replaced by abscesses (**Fig 16**) of numerous neutrophils, plasma cells and lymphocytes (**Fig17**) besides basophilic thick elongated hyphae-like and round bodies among the inflammatory cells (**Fig 18**). These bodies may be represented to some yeast secondary infection. The inflammation was extended into the muscular layer and surrounded the ureter.

Kidneys:

Bacterial Isolates:

Staphylococcus aureus and *Escherichia coli* were isolated from the kidneys as mixed infection.

Pathological Findings:

Macroscopically, multiple stones of variable sizes and shapes (1-3 cm in diameter) were detected with numerous tiny cysts in the medulla and cortex. These calculi showed smooth surfaces and were solid and friable. Microscopically, the renal tubules were cystically dilated and lined by flattened epithelium. The surrounding tubules were atrophied and the others were necrotic and surrounded by fibrous connective tissue (**Fig 19**). Focal interstitial aggregations of round cells and extravasated erythrocytes were noticed (**Fig 20**). Almost all the renal tubules at the medulla were dilated or atretic (**Fig 21**). Some dilated renal tubules showed light eosinophilic laminated material surrounded with vascular connective tissue (**Fig 22**). Coagulative necrosis in renal tubular epithelia and aggregation of round cells were focally observed (**Fig 23**). Such inflammatory infiltrates (lymphocytes, plasma cells and macrophages) were also seen in the medulla and around the renal pelvis (**Fig 24**). Extensive fibrosis were diffusely replaced the medulla and the remaining renal tubules were necrotic (**Fig 25**). The renal pelvis was focally necrotic and the surrounding tubules were stuffed with vacuolated eosinophilic proteinaceous material (**Fig 26**). Basophilic granular mineralization was focally replaced the renal pelvis and medulla and enclosed in fibrous connective tissue (**Fig 27**). Most of the glomeruli showed hyperplasia in the glomerular tuft and dilated Bowman's space with eosinophilic material (**Fig 28**).

Table (1): Incidence of isolated pathogens from urinary tracts of buffalo calves.

Isolates	Ureters		Urinary bladders		Kidneys	
	No	%	No	%	No	%
Staphylococcus aureus	5	25	6	30	0	0
Enterococcus faecalis	3	15	2	10	0	0
Escherichia coli	2	10	3	15	0	0
Klebsiella pneumoniae	0	0	1	15	0	0
Mixed infections:						
E.faecalis+Staph.aureus	2	10	0	0	0	0
E.faecalis+Klebsiella sp	1	5	3	15	0	0
Staph. Aureus+E.coli	1	5	0	0	2	20
E.coli+Coryne.spp.	1	5	0	0	0	0
Negative samples	5	25	5	25	8	80
Total	20	100	20	100	10	100

No=Number. %= Percentage.

Table (2): The prevalence of the virulence factors among the *Enterococcus faecalis* isolates.

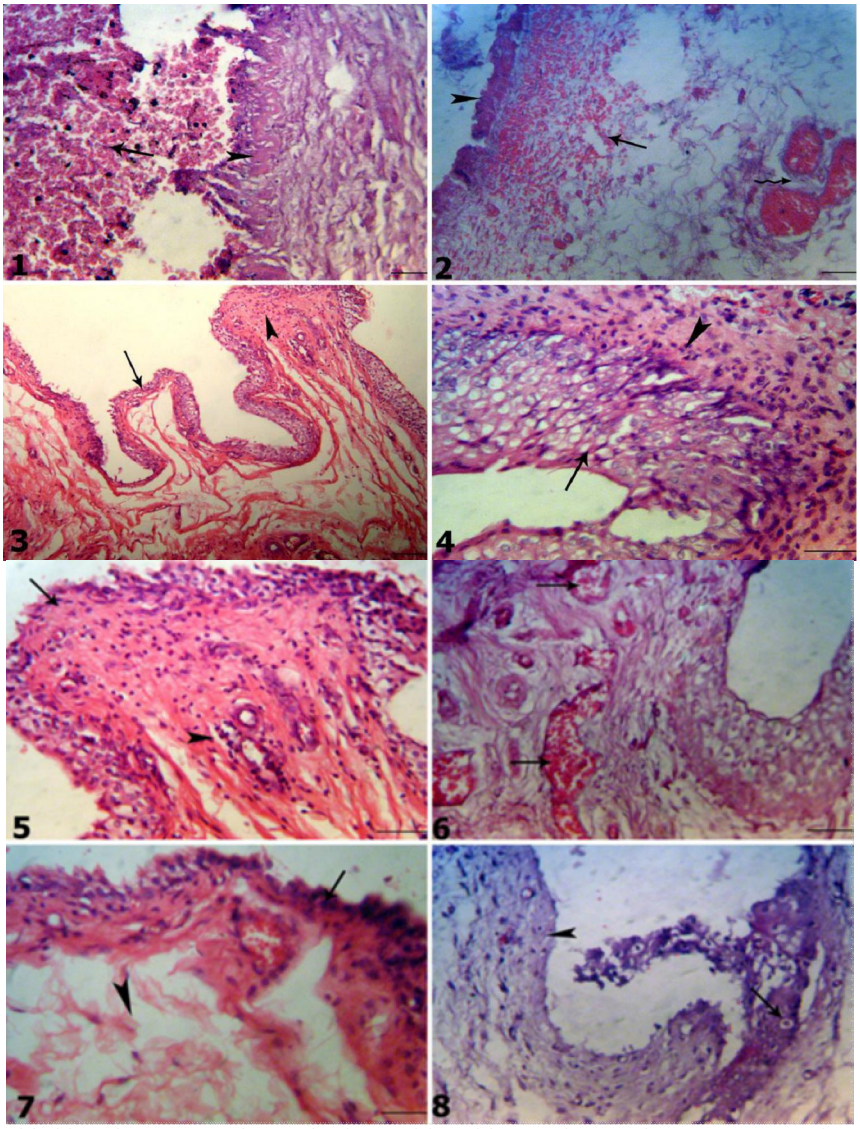
Virulence factors	Positive isolates for virulence factors	
	No	%
Haemolysin factor	3/8	37.5
Gelatin production factor	4/8	50
Aggregation factor	1/8	12.5
Biofilm formation factor	6/8	75

No=Number. %=Percentage.

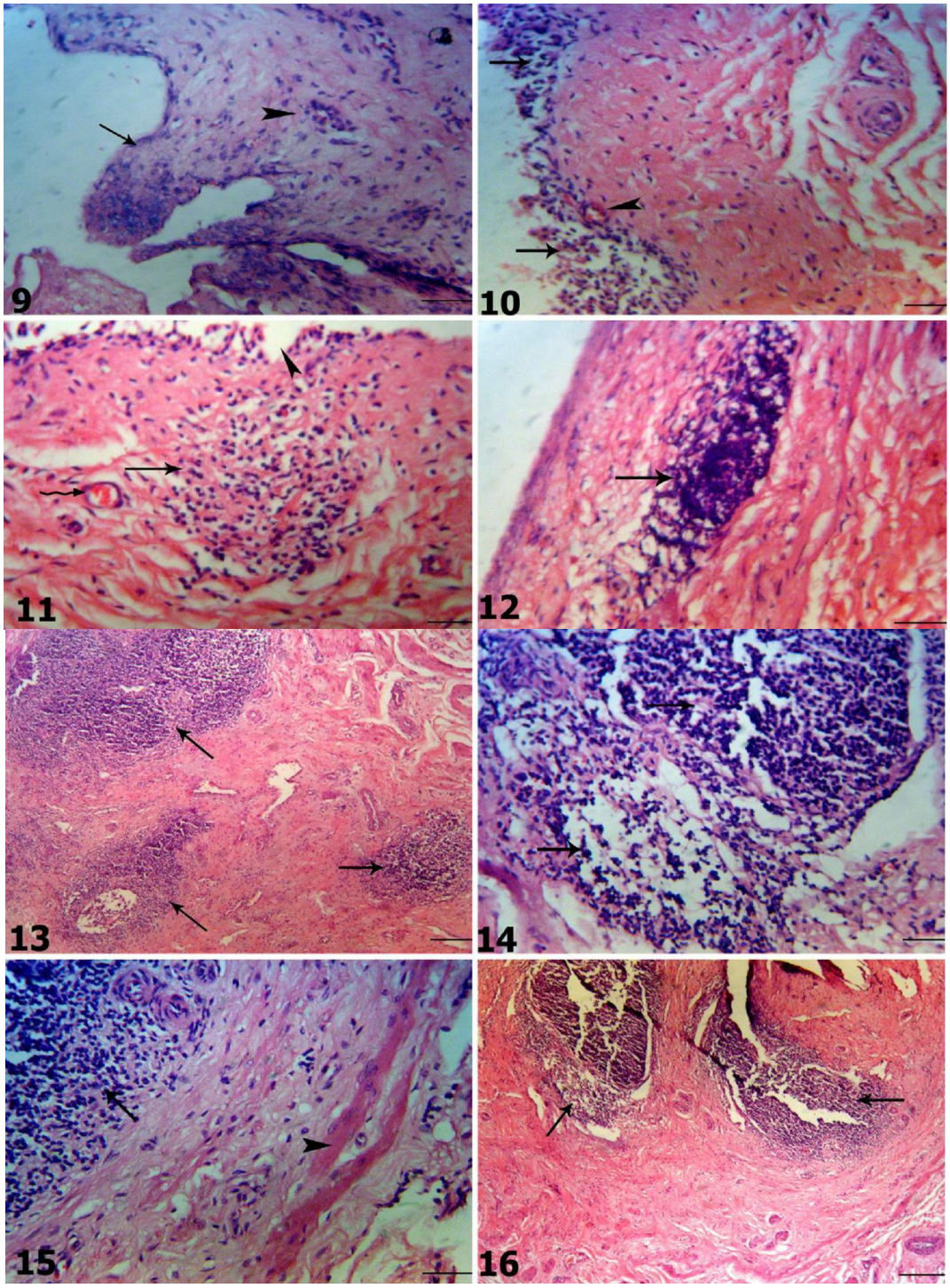
Table (3): Antibiogram of the isolated organisms from urinary tracts of buffalo calves.

Antibiotic	*Staph. aureus*	*E. faecalis*	*E. Coli*	*K. pneumoniae*
Ampicillin	-	-	-	+
Tetracycline	-	-	+	+
Colistin	-	-	+++	+++
Streptomycin	+++	+++	+++	+++
Gentamycin	+++	+++	+++	+++
Ofloxacin	++	++	+++	+++
Ciprofloxacin	+	+	+++	+++
Amikacin	+++	+++	+++	+++

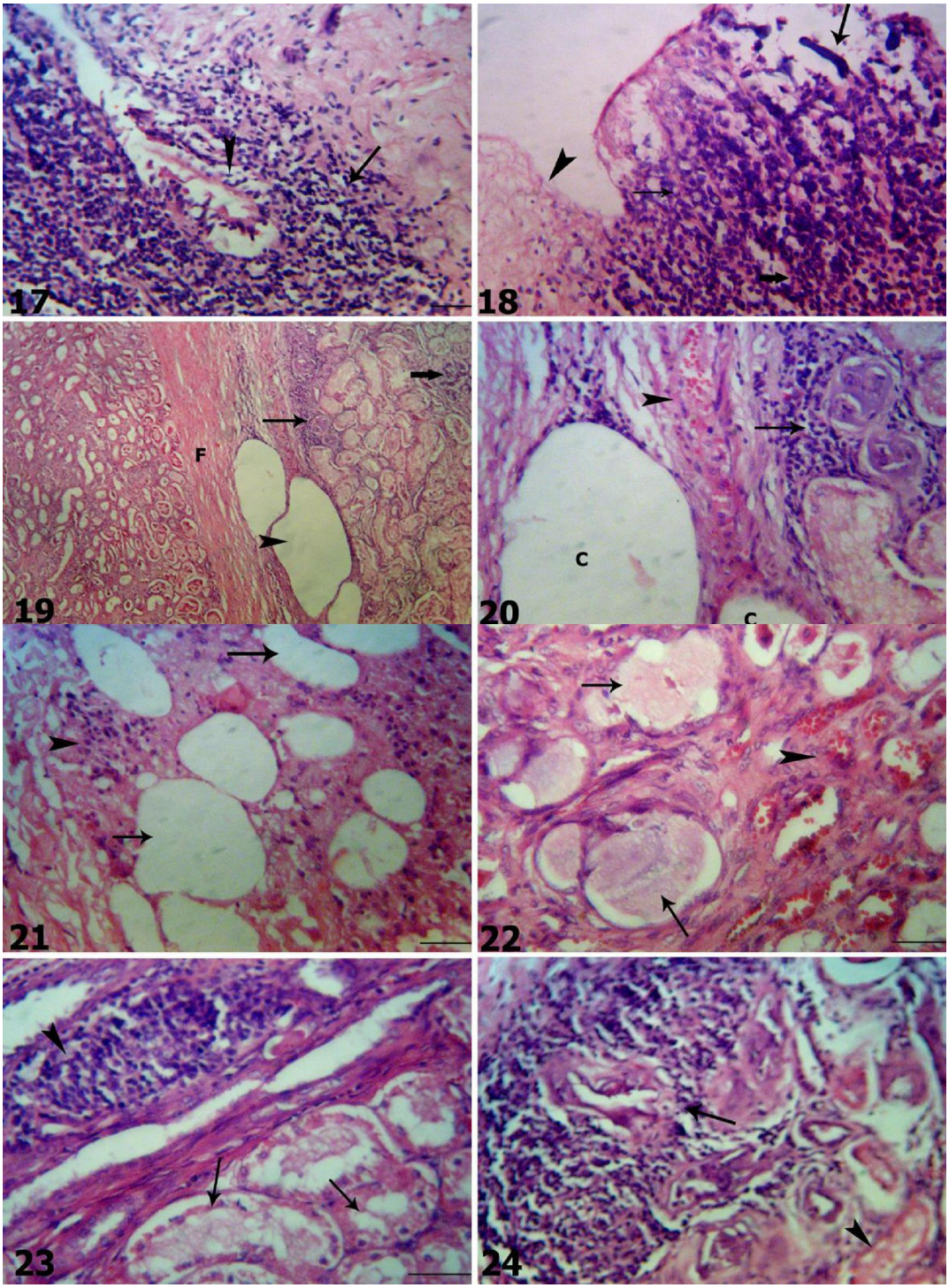
(0-30 %) = -, (35-45) =+, (65-75%) =++, (75-100 %) =+++



Figs (1-8): Urinary bladder show Acute hemorrhagic cystitis with massive erythrocytes aggregation (arrow) inside the lumenH&E×120 (1) and in the submucosa besides necrotic mucosa (arrowhead), marked congested blood vessels and few leukocytes infiltrationH&E ×120 (2). Papillary hyperplasia in the lining epithelium (arrow) H&E×120 (3) with hydropic degeneration and few neutrophils infiltration in the subepithelial zone (arrowhead)H&E ×200(4). Focal area of epithelial denudation (arrow) with and leukocytes infiltration (arrowhead)×200 (5). Mild cystitis with severe congested submucosal blood vessels (arrow) and edema H&E×200(6). The edema became prominent under focal necrotic mucosa (arrowhead)H&E×200 (7). Focal ulceration (arrowhead) with adjacent hyperplastic mucosal epithelia containing Malakoplakia inclusions (arrow)H&E×200 (8).

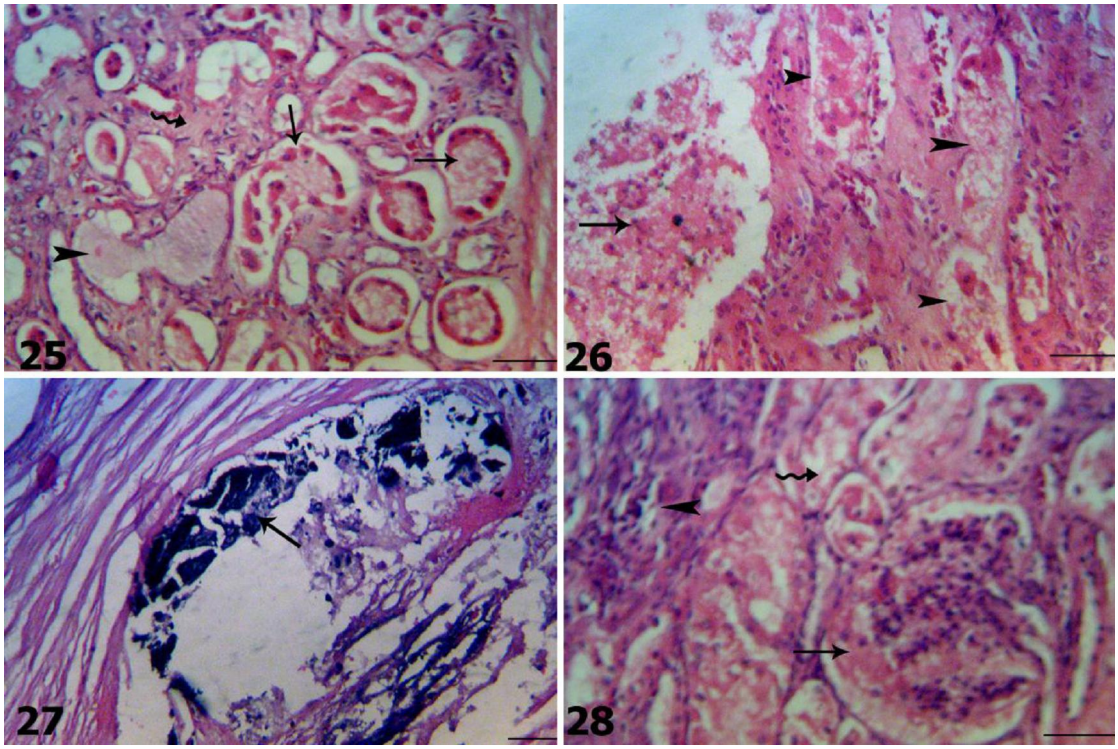


Figs (9-16): Urinary bladder shows sloughing the epithelial lining (arrow) and edema in lamina propria with few leukocytes infiltration (arrowhead) H&E ×120(9). Focal replacements of the mucosa with mononuclears (arrows) H&E×200 (10) which invaded to the lamina propria and submucosa (arrow) H&E×200 (11) besides desquamation and necrosis in the lining epithelia. Chronic cystitis with proliferated submucosal lymphocytes forming follicular aggregation (arrow) H&E×200 (12).Multifocal abscesses in the submucosa (arrow) H&E×120 (13) with huge numbers of neutrophils and round cells (arrow) H&E ×200(14) and degenerated smooth muscles (arrowhead) H&E×200 (15). Necrotic mucosa is replaced by abscesses (arrow) H&E×120 (16).



Figs (17-18): Urinary bladder shows necrotic mucosa (arrowhead) replaced with neutrophils and round cells (arrow)H&E×200 (17) besides basophilic thick elongated hyphae-like and round bodies among the inflammatory cells (arrow)H&E×200 (18).

Figs (19-24): Kidney shows cystically dilated (C) and necrotic renal tubules surrounded by fibrous connective tissueH&E×120 (19) with round cells aggregation (arrow) and extravasatederythrocytesH&E (19,×200 20). Medulla with cystic dilation (arrow) or atresia of the renal tubules (arrowhead)H&E×200 (21). Renal tubules containing light eosinophilic material (arrow) surrounded with vascular connective tissue (arrowhead)H&E×200 (22). Coagulative necrosis in renal tubular epithe(arrow) and aggregation of round cells (arrowhead) H&E×200(23). Renal medulla replaced with aggregation of lymphocytes, plasma cells and macrophages (arrow)H&E×200 (24).



Figs (25-28): Kidney shows
Fibrotic medulla with necrotic (arrow) and dilated the remaining renal tubules H&E ×200(25). Severe necrosis in the renal pelvis (arrow) and adjacent medulla with vacuolated eosinophilicproteinaceous material inside the renal tubule (arrowhead) H&E×200 (26). Basophilic granular mineralization (arrow) focally replaced the renal pelvis and medulla H&E ×200(27). Hyperplasia in the glomerular tuft and dilated the Bowman's space with eosinophilic material (arrow) H&E ×200(28).

4. Discussion

Although the bladder and ureters are often exposed to bacteria and although urine generally supports bacterial growth, the combined effects of bladder emptying by urination and an intrinsic defense mechanism associated with bladder epithelium assist in resisting bacterial infection of the bladder and other urinary tract **(Cox and Hinman, 1961 and Nassan, 2009). Parry (2005**) explained that the renal medulla is very susceptible to infection due to its relatively poor blood supply. Additionally its interstitium has a high osmolality that inhibits neutrophil function, and a high ammonia concentration that complement activation. Consequently, upon bacterial colonisation of the renal pelvis, the medulla is readily infected. Organisms ascend within the collecting duct system leading to tubular epithelial necrosis and inflammation that progressively extends throughout the tubular system and interstitium, radiating from pelvis to cortex.in the present study the most common isolates from urinary tracts (50 samples) of buffalo calves were *staphylococcus aureus* (22%), *enterococcus faecalis* (10%), *Eschirichia coli* (10%) and *klebsiella pneumonie* (2%) as single infections, while the mixed infections were *(E.faecalis+Staph*) (4%), (*E.faecalis+Klebsiella spp.)* (8%), (*Staph.+E.coli*) (6%), and (*E.coli+Coryne*) (2%) and the negative samples for bacteriological examinations were (36%). **(Jombo *et al.*, 2011**) isolated the same pathogens from the urinary tracts of humans but in a lower percentage and the same pathogens isolated from the kidneys of cattle and buffaloes in a lower percentage by **(Ibrahim *et al.*, 2008).** in our present study the staph. aureus was one of the causes of acute cystitis, chronic suppurative cystitis and nephritis, and these pathological finding were due to that S. aureus expresses many potential virulence factors: (1) surface proteins that promote colonization of host tissues; (2) invasins that promote bacterial spread in tissues (leukocidin, kinases, hyaluronidase); (3) surface factors that inhibit phagocytic engulfment (capsule, Protein A); (4) biochemical properties that enhance their survival in phagocytes (carotenoids, catalase production); (5) immunological disguises (Protein A, coagulase); (6) membrane-damaging toxins that lyse eucaryotic cell membranes (hemolysins, leukotoxin, leukocidin; (7) exotoxins that damage host tissues or otherwise provoke symptoms of disease (SEA-G, TSST, ET); and (8) inherent and acquired resistance to antimicrobial

agents**. (Robert *et al.,* 2006). (Khamis *et al.,* 2009)** Screened for urinary bladder affections in camels, the bacteriological examination revealed isolation of *Staphylococcus sp*., *Corynebacterium sp.* and *E. coli sp*. from the camel's urine. According to the histopathological findings, camels under investigation were classified into acute cystitis and chronic cystitis, we in partial agreement with the authors work.

Enterococci are important causes of urinary tract infections, bacteremia, intra-abdominal infections and endocarditis, in our present work *E. faecalis* found to be the cause of acute and chronic cystitis. The virulence of enterococcus faecalis is due their adherence and lytic activity. Enterococci must first be able to colonize the mucosal surfaces and then cause the infection. After the colonization, bacteria produce pathological changes in the host through direct toxic activity, or indirectly by inducing an inflammatory response. Some markers have been proposed as possible virulence factors in enterococci. Haemolysin, gelatinase (cazeinase), entero-coccal surface protein (esp) and aggregation substance are the most frequently mentioned virulence determinants **(Coque *et al.,* 1995, Coburn *et al.,* 2004).**We suggested that the above causes were be considered in the presence of those pathological findings.Our results go mostly hand in hand with **Eman *et al.* (2011)** who screened the bacteriological, pathological and biochemical changes associated with the urinary tract affections in cattle and buffaloes Bacterial isolated strains were, *E coli*, Proteous sp, *Streptococcus feacalis*, *Staphylococcus aureus*, Klebsilla, and Salmonela spp. According to the histopathological findings, the animals under investigation were suffered from pyelonephritis, uretritis and cystitis. Also **Ibrahim *et al.*,(2008)** recorded cases of pyelonephritis in kidneys of buffaloes which caused by nearly the same pathogens that we isolated in the present study.

Uropathogenic *Escherichia coli* (UPEC) is a causative agent in the vast majority of urinary tract infections (UTIs), including cystitis and pyelonephritis, and infectious complications, which may result in acute renal failure in healthy individuals as well as in renal transplant patients. UPEC expresses a multitude of virulence factors to break the inertia of the mucosal barrier **(Justyna *et al.,* 2012).**Malakoplakia is an inflammatory disorder of unknown cause,these inflammatory lesions are characterized by apredominance of histocytes,the so- called Van Hansmann cells with diagnostic intracytoplasmic inclusion known as Michaelis-Gutmann bodies.These are commonly associated with urinary tract infection caused by E.coli. and the urinary bladder is the single most common site of occurrence of those lesions **(Petersen *et al.,* 2009).** *Escherichia coli* was resbonsible for the acute and chronic cystitis as well as nephritis in buffalo calves in our study. **Daoust (2011)** described cystitis as inflammation of the urinary bladder which is generally due to bacterial infections, formation and accumulation of uroliths, and, to a lesser extent, exposure to toxic compounds, the bacteria involved in cystitis are similar to those causing pyelonephritis: *Corynebacterium renale* in cattle; *E. coli*, *Proteus* species*, Enterobacter* species, *Pseudomonas aeruginosa*, we in partial agreement with his study.

In our present study *Corynebacterium spp*.in mixed infection with *E.coli* was responsible for the chronic cystitis and nephritis. **Saba,(2010)** reported that *C. renale* type I has ability to produce kidney damage after 48 hr. post inoculation revealed emboli glomeruler nephritis with less number of *C. renale*, also there is infiltration of polymorphnuclear inflammatory cells and nephrosis, in addition to vacuolar degeneration, coagulative necrosis with blood vessel congestion, our study was partially agree with her**. Stevens *et al.*, (2007**) described the most prominent histologic lesion in two female Macaques due to *Corynebacterium renale* which was transmural necrohemorrhagic to fibrinosuppurative cystitis that was diffuse and severe. Corynebacterial necrohemorrhagic cystitis therefore was determined to be the cause of death in both animals. Our study was disagree with those results.

In the present work the prevalence of virulence factors have been studied and we found that the most prevalent virulence factor among the enterococci was the biofilm (enterococcus surface protein) which was (75%), this result is similar to **(Sharma *et al.,* 2012)**. The incidence of Haemolysin was (37.5%) and gelatiase was (50%) this agree with **(Jankoska *et al.,* 2008**) which found that Haemolysine actively occurred in up to 60% of isolates from urine samples, while gelatinase found in 68% of isolates in the urine samples. Gelatinase and haemolysin producing strains of *Enterococcus faecalis* have been shown to be virulent in animal models. Gelatinase is a protease produced by enterococci that is cabable of hydrolysin gelatin, collagen, casein and other peptides. Haemolysin causes rupture of variety of target membranes, including bacterial cells, erythrocytes and other somatic cells with haeomolytic activity on blood agar (**Archimbaud *et al.,* 2002; Vergis *et al.,* 2002; and Sharma *et al.,* 2012).** Haemolysin is a citolytic protein that lyses human, horse and rabbit erythrocytes and also has a bactericide activity against many Gram-positive bacteria. Strains enriched with this protein have been found among enterococci associated with an increased severity of infection **(Vergis *et al.,* 2002, Roberta Creti *et al.,* 2004)**.Enterococci are intrinsically resistant to many antibiotics. Unlike

acqui-red resistance and virulence traits, which are usually transposon or plasmid encoded, intrinsic resistance is based on chromosomal genes, which are non-transferable. Penicillin, ampicillin, piperacillin, imipenem, and vancomycin are among the few antibiotics that show inhibitory, but not bactericidal, activity against *E. faecalis. E. faecium* is less susceptible to ß-lactam antibiotics than *E. faecalis* due to the lower affinities of their penicillin-binding proteins for anti-biotics **(Mundy *et al.*, 2000).** A review of the antimicrobial sensitivity profile of the bacterial isolates from urinary tract samples of buffalo calves in our study should that the most active drugs against majority of the bacterial isolates where colistin, stryptomycin, ofloxacin, ciprofloxacin (65 to 100%), our results were in agreement with **(Jombo *et al.*, 2011).**

Based on the current study, it could be concluded that the bacteriological and pathological changes associated with the urinary tract affections in buffalo calves, the most frequent isolates from the urinary tract of buffalo calves were the *Enterococcus faecalis, Staphylococcus aureus, Eschirichia Coli, Corynebacterium spp.* and *Klebsiella spp.* and the virulence of enterococci in general is due to their adherence and lytic activity. According to the histopathological findings, the animals under investigation were suffered from nephritis, nephrolithiasis and cystitis, Finally, ofloxacin, gentamycin, ciprofloxacin and stryptomycin could be considered for treatment of urinary tract affection.

Figs (1-8): Urinary bladder show Acute hemorrhagic cystitis with massive erythrocytes aggregation (arrow) inside the lumen H&E×120 (1) and in the submucosa besides necrotic mucosa (arrowhead), marked congested blood vessels and few leukocytes infiltration H&E ×120 (2). Papillary hyperplasia in the lining epithelium (arrow) H&E ×120 (3) with hydropic degeneration and few neutrophils infiltration in the subepithelial zone (arrowhead) H&E ×200(4). Focal area of epithelial denudation (arrow) with and leukocytes infiltration (arrowhead) H&E ×200 (5). Mild cystitis with severe congested submucosal blood vessels (arrow) and edema H&E ×200(6). The edema became prominent under focal necrotic mucosa (arrowhead) H&E ×200 (7). Focal ulceration (arrowhead) with adjacent hyperplastic mucosal epithelia containing Malakoplakia inclusions (arrow) H&E ×200 (8).

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