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ORIGINAL ARTICLE

# The linkage between prevalence of integron I and reduced susceptibility to biocides in MDR *Klebsiella pneumoniae* isolated from neonates

**Pola Samir[1], Ahmed Mohamed El-Baz[2*], Hany Ibrahim Kenawy[1]**

*[1]Department of Microbiology and Immunology, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt*
*[2]Department of Microbiology and Immunology, Faculty of Pharmacy, Delta University for Science and Technology, Gamasa 11152, Egypt*

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## ABSTRACT

**Background and Objectives:** *Klebsiella pneumoniae* causes challenging nosocomial fatal infections including neonatal sepsis. Our study aims at clarifying the contribution of integrons in the observed reduced susceptibility of multidrug-resistant (MDR) *K. pneumoniae* isolated from septicemic neonates to the clinically used antimicrobial agents and biocides.
**Materials and Methods:** Eighty-six *K. pneumoniae* isolates were collected from Mansoura University Children's Hospital from septicemic neonates. Isolates were subjected to antibiotic and biocide susceptibility using disk diffusion and the agar dilution method, respectively. The distribution of different classes of integrons was screened in the isolates by PCR. Detected inegron I was sequenced in selected isolates.
**Results:** Fifty-seven isolates (66.27%) were MDR. In the MDR isolates, class I integron was detected in 23 (40.3%), integron III was detected in 20 (35%), whereas integron II could not be detected. Sequencing results of integron I from MDR *K. pneumoniae* isolates revealed that only aminoglycoside and folate synthesis inhibitors gene cassettes were detected, while the rest of the resistance genes were not associated with integron I.
**Conclusion:** The presence of integron I in MDR *K. pneumoniae* tested isolates may contribute only to some biocide resistance, however, it does not seem to be the only contributor in multiple drug resistance.

**Keywords:** *Klebsiella pneumoniae*; Integrons; Disinfectants; Drug resistance; Newborn

## INTRODUCTION

In underdeveloped and developing nations, Gram-negative bacteria are the most common cause of newborn sepsis (1). *Klebsiella pneumoniae* represents an important pathogen that causes nosocomial infections and subsequently neonatal sepsis (2). Neonatal sepsis represents a major cause of mortality among neonates. Neonatal sepsis cause one million death per year according to WHO statistics (3, 4). *K. pneumoniae* is responsible for the majority of neonatal sepsis, especially in developing countries (5).

The challenge of *K. pneumoniae* stems from its ability to acquire resistance against many antimicrobial agents including biocides (6). There are many mechanisms through which the pathogen can acquire resistance against antimicrobial agents. Mobile genetic elements including integrons are considered the major factor in the dissemination of multidrug resistance (MDR) (7). As a result of acquiring resis-

*Corresponding author: Ahmed Mohamed El-Baz, Ph.D, Department of Microbiology and Immunology, Faculty of Pharmacy, Delta University for Science and Technology, Gamasa 11152, Egypt. Tel: +2-0106-9096934 Fax: +2-050-2770145 Email: ahmed.elbaz@deltauniv.edu.eg

tance to at least one agent in three or more antimicrobial groups, MDR was coined (8). According to their genomic context, integrons can be found as mobile integrons when they are related to mobile DNA elements such as insertion sequences, transposons, conjugative plasmids, and as chromosome integrons when they are found in bacterial chromosomes (9). The risk of multiple resistance of *Klebsiella* infection is exaggerated, which leads to an increase in hospital stay time and also poses difficulty for the medical team to choose the best treatment (9, 10). There is a possible linkage between biocides resistance and antibiotic resistance established on the presence of mobile genetic elements (11). Integrons are recombination and expression mechanisms that can grab exogenous gene cassettes and recombine them (9). Furthermore, integrons may play an important role in the pathogenicity of microorganisms, as well as enable the transfer of virulence factors among different bacteria. According to the reports available, integrons have a wide distribution among clinically isolated bacteria; also, their mobility has become a major problem in antibiotic resistance in clinical specimens (2). Class I, II, and III integrons represent the most predominate classes that confer multiple resistance in Gram-negative bacteria including *Enterobacteriaceae* (12). Controlling the nosocomial infections in health care institutes depends mainly on using different biocides either disinfectants for non-living surfaces or antiseptics for living surfaces (11). Glutaraldehyde is a disinfectant and a sterilant aldehyde. The bactericidal action of glutaraldehyde might be attributed to the denaturation of proteins and nucleic acids through alkylation; the reaction is irreversible at the level of nucleic acids (13). Chloroxylenol (Dettol) is a potent chlorinated phenolic disinfectant, antiseptic and bactericide. Chloroxylenol acts as a bactericide by distrusting the cell membrane of bacteria (14). Povidone-iodine is a pharmaceutical preparation made up of elemental iodine, hydrogen iodide, and povidone (15). It kills bacteria by releasing iodine, which causes lipids to be iodinated and cytoplasmic and membrane components to be oxidized (15). The extensive misuse of biocides in hospitals led to the emergence of resistance among many bacteria, especially those that have the ability to acquire resistance (6).

The current study aimed at determining the resistance profile of *K. pneumoniae* isolated from neonates in Mansoura University Children's Hospital against both antimicrobial agents and biocides. Furthermore, to layout the possible correlation between the presence of integrons among MDR *K. pneumoniae*, being resistant to three or more classes of antimicrobial agents and the reduced susceptibility of these isolates to different clinically used antimicrobial agents.

## MATERIALS AND METHODS

**Bacterial isolates.** Eighty-six *K. pneumoniae* isolates were collected from neonatal sepsis through blood samples from Mansoura University Children's Hospital, Egypt. The isolates were identified through culturing on nutrient agar media and MacConkey's agar (Oxoid, UK.). The characteristic formed colonies of *K. pneumoniae* were subjected to typical biochemical tests including TSI (Triple Sugar Iron Agar), indole, methyl Red (MR), Voges–Proskauer (VP), citrate (C), and motility test.

**Antibiotic susceptibility testing.** The antibiogram of *K. pneumoniae* isolates was determined using different clinically available antimicrobial agents according to the Modified Kirby-Bauer method (16). The antibacterial agents used were; cefoperazone/sulbactam (CES, 75/30 µg), ceftazidime (CAZ, 30 µg), cefepime (FEP, 30 µg), imipenem (IPM, 10 µg), colistin (CT, 10 µg), ofloxacin (OFX, 5 µg), gatifloxacin (GAT, 5 µg), norfloxacin (NOR, 10 µg), amikacin (AK, 30 µg), sulfamethoxazole/trimethoprim (SXT, 1.25/23.75 µg), aztreonam (ATM, 30 µg), ampicillin/sulbactam (SAM, 20 µg), piperacillin (PRL, 100µg), ceftriaxone (CRO, 30 µg), and nitrofurantoin (F, 300 µg). All of the used antimicrobial disks were purchased from Oxoid (Hampshire, England). As suggested by CLSI, the inoculum was optimized to 0.5 McFarland turbidity standard and inoculated on Mueller Hinton Agar (Oxoid, Hampshire, England). After adding the antimicrobial discs, the plates were incubated at 37°C for 18 hours. The inhibitory zones were measured, and the results were interpreted using CLSI criteria (2019) (17).

**Biocide susceptibility test.** Three different biocides were tested to determine the minimum inhibitory concentrations (MIC) by the agar dilution method (18). The following biocides were tested: glutaraldehyde (El-Nasr Pharmaceutical Company, Cairo, Egypt), betadine (Iodine-povidone complex 10%)

(El-Nile Pharmaceutical Company, Cairo, Egypt), and Dettol (4.8% chloroxylenol) (Royal cosmetic Co., Egypt), starting with the concentrations previously recommended. All biocides were added to the sterilized Mueller–Hinton agar medium at a temperature between 50-55°C (11) to obtain concentration ranges: 500-4000 mg $L^{-1}$ (5-40 mmol) for glutaraldehyde, 100-800 mg $L^{-1}$ (0.35-2.8 mmol) for betadine, and 10-80 mg $L^{-1}$ (0.02-0.16 mmol) for Dettol.

**Colony PCR for detection of class I, II, and III integrons.** DNA was extracted by colony PCR method for each MDR *K. pneumoniae* isolate (19). A pure colony of *K. pneumoniae* strains was suspended in 200 µL of sterile deionized water and incubated at 100°C for 10 min. After centrifugation at 12,000 rpm for 2 min, the supernatant was used as a template DNA and stored at - 20°C until use.

Each PCR reaction consisted of 12.5 µL of MyTaq Red Mix (Bioline Co., UK), 1 µL of each primer (10 µM each, see Table 1), 2.5 µL DNA template, and nuclease-free water to the final volume (25 µL). PCR reaction was performed under the following thermal cycling conditions, denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing temperature as indicated in Table 1 for 30 s, extension at 72°C for 1 min and final extension at 72°C for 7 min.

**Sequencing of Integron-I positive isolates.** Six integron I positive amplicons representing various MDR *K. pneumoniae* isolates were chosen, purified, and sequenced to evaluate the relationship between integron I prevalence and antibiotic/biocide resistance. QIAquick PCR Purification (Qiagen, Hilden, Germany) was used to clean up PCR amplicons according to the manufacturer's instructions. The concentration and purity of eluted DNA was determined using NanoDropTM 2000/2000c Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Purified PCR amplicons were sent for sequencing from both directions using the forward and reverse primers intended to amplify integron I (Colors Medical Labs, Cairo, Egypt). Sequenced products were analyzed using the BLAST database (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

**Statistical analysis.** The susceptibility pattern of tested *K. pneumoniae* isolates against different antimicrobials agents in association to presence or absence of Integron I and Integron III were analyzed using Fisher's exact test. Statistical analysis was carried out using GraphPad Prism (version 9.3.1). Significance was accepted at ($P < 0.05$).

## RESULTS

**Identification of *K. pneumoniae* isolates.** *K. pneumoniae* isolates were confirmed among 86 isolates out of 257 clinical specimens. They were identified through the characteristic morphology of pink mucoid colonies on MacConkey agar. All *K. pneumoniae* isolates were confirmed as, lactose fermenter, A/A with TSI, indole negative, MR negative, VP positive, citrate positive, and motility negative Gram-negative bacilli.

**Antimicrobial susceptibility pattern of *K. pneumoniae*.** Susceptibility patterns to the tested antimicrobial agents are shown in Figs. 1 and 2. The antimicrobial susceptibility data revealed that 57 (66.27%) isolates were MDR. High resistance percentage was detected among cephalosporins with resistance rates of 56.9%, 66.3%, 69.8%, and 88.4% for cefepime, cefoperazone/sulbactam, ceftriaxone, and ceftazidime, respectively. The highest resistance was detected for ampicillin/sulbactam (98.8%). Moderate resistance was detected for antimicrobial agents of the fluoroquinolones and

**Table 1.** Primers used for integrons detections.

| Target Sequence | Primer | Sequence 5`-3` | Annealing Temp. | Product size | Ref. |
|---|---|---|---|---|---|
| Integron I | hep58F-18 | TCATGGCTTGTTATGACTGT | 55°C | Variable | (20) |
| | Hep59R-18 | GTAGGGCTTATTATGCACGC | | | |
| Integron II | intI2L F-18 | CACGGATATGCGACAAAAAGGT | 57°C | Variable | (21) |
| | intI2RR-18 | GTAGCAAACGAGTGACGAAATG | | | |
| Integron III | intI3L F-18 | GCCTCCGGCAGCGACTTTCAG | 58°C | Variable | |
| | intI3RR-18 | ACGGATCTGCCAAACCTGACT | | | |

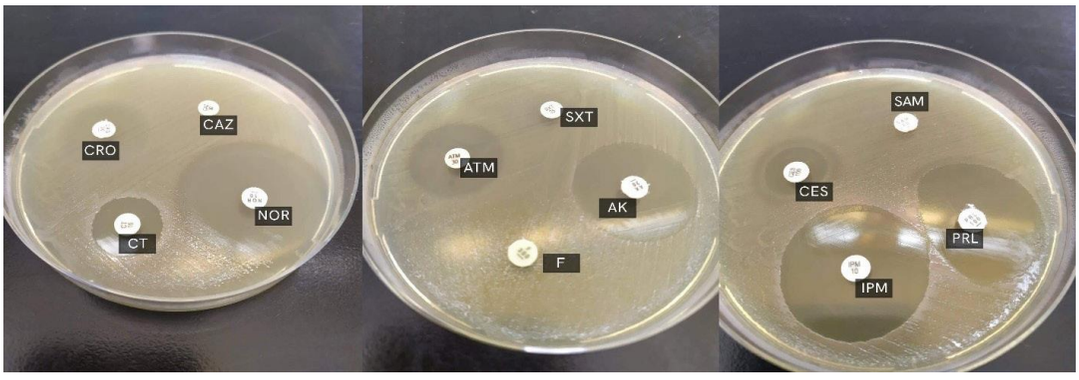
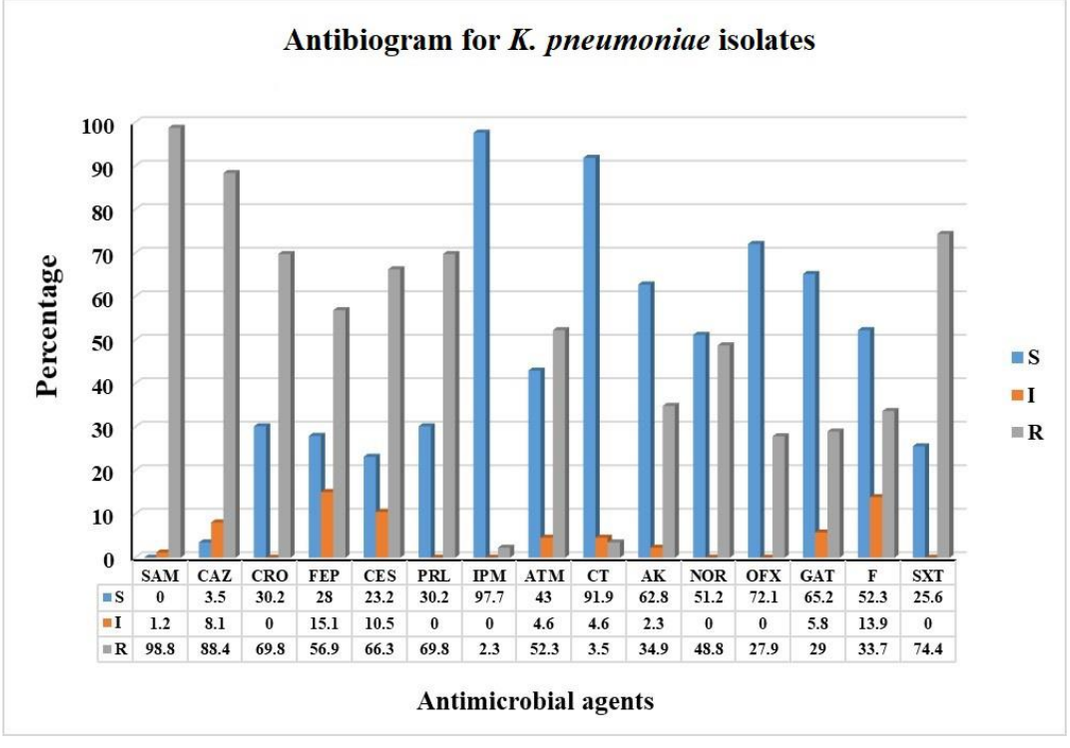



**Fig. 1.** The antimicrobial susceptibility of clinical *K. pneumoniae* isolates to different antibiotics by disk diffusion method.




| | SAM | CAZ | CRO | FEP | CES | PRL | IPM | ATM | CT | AK | NOR | OFX | GAT | F | SXT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S | 0 | 3.5 | 30.2 | 28 | 23.2 | 30.2 | 97.7 | 43 | 91.9 | 62.8 | 51.2 | 72.1 | 65.2 | 52.3 | 25.6 |
| I | 1.2 | 8.1 | 0 | 15.1 | 10.5 | 0 | 0 | 4.6 | 4.6 | 2.3 | 0 | 0 | 5.8 | 13.9 | 0 |
| R | 98.8 | 88.4 | 69.8 | 56.9 | 66.3 | 69.8 | 2.3 | 52.3 | 3.5 | 34.9 | 48.8 | 27.9 | 29 | 33.7 | 74.4 |

* R: resistant – I: Intermediate resistant – S: sensitive.

**Fig. 2.** Antibiogram pattern of *K. pneumoniae* isolates against antimicrobial agents.

aminoglycosides with resistance rates of 27.9%, 29%, 34.9%, and 48.8% for ofloxacin, gatifloxacin, amikacin, and norfloxacin, respectively. The lowest resistance was detected for colistin and imipenem with resistance prevalence of 2.3 and 3.5%, respectively.

**Biocide sensitivity.** The MICs of three different biocides were determined for all *K. pneumoniae* isolates. In case of MDR isolates, 0.8 mg/ml of glutaraldehyde was inhibitory to all MDR isolates (the $MIC_{50}$ was 0.55). For betadine, 4 mg /ml inhibited the growth of all MDR isolates ($MIC_{50}$ was 1.4), while in case of Dettol, 1.6 mg /ml inhibited the growth of all MDR isolates ($MIC_{50}$ was 1.3).

**Integron prevalence.** PCR amplification of the integron I variable region revealed that only 23 (40.3%) of the MDR isolates were integron I positive. For integron II, no MDR isolates were integron II positive. In case of integron III, only 20 of the MDR isolates (35%) were integron III positive as shown in Fig. 3.

The presence of different classes of integrons, re-

**Table 2.** Incidence of integrons, resistance genes, and reduced susceptibility to biocides in MDR *K. pneumoniae* isolates

| Isolate No. | Integron type (bp) | Disinfectant resistance profile | Antibiotic resistance profile |
|---|---|---|---|
| 1 | I (1500) | Dettol, GLU | PRL, SAM, CAZ, CRO, FEP, CES, IPM, ATM, NOR, AK, SXT, F |
| 2 | III (300) | Dettol, GLU | SAM, CAZ, FEP, NOR, OFX, GAT, AK, SXT, F |
| 5 | III (300) | Dettol, GLU | PRL, SAM, CAZ, FEP, CES, OFX, GAT, SXT |
| 6 | -- | Dettol, GLU | PRL, SAM, CAZ, CRO, CES, ATM, NOR, AK, SXT, F |
| 7 | I (800) | PVP- I, Dettol | PRL, SAM , CAZ, CRO, FEP, CES, ATM, NOR, OFX, GAT, SXT |
| 10 | -- | GLU | PRL, SAM, CAZ, CRO, FEP, CES, ATM, AK, SXT |
| 13 | I (1500) & III (300) | Dettol | PRL, SAM, CAZ, CRO, FEP, CES, ATM, AK, NOR, SXT , F |
| 14 | -- | PVP- I, Dettol, GLU | PRL, SAM, CAZ, CRO, FEP, CES, ATM, NOR, OFX, GAT, SXT, F |
| 15 | I (250) & III (400) | Dettol | SAM, CAZ, CRO, CES, SXT, F |
| 16 | -- | Dettol | PRL, SAM, CAZ, CRO, CES, ATM, NOR, SXT, F |
| 18 | I (1000) | -- | PRL, SAM, CAZ, CRO, FEP, CES, ATM, NOR, OFX, GAT, AK, SXT, F |
| 19 | -- | Dettol, GLU | SAM, CAZ, CRO, FEP, CES, NOR, AK, SXT, F |
| 20 | -- | PVP- I, Dettol, GLU | PRL, SAM, CAZ, CRO, FEP, CES, ATM, CT, NOR, OFX, GAT, AK, SXT, F |
| 21 | -- | -- | PRL, SAM, CAZ, CRO, FEP, CES, ATM, AK, SXT |
| 22 | I (250) & III (500) | Dettol | PRL, SAM, CAZ, CRO, FEP, CES, ATM, NOR, OFX, GAT, AK, SXT, F |
| 23 | I (800) | -- | PRL, SAM, CAZ, CRO, FEP, CES, ATM, CT, SXT |
| 24 | I (2000) & III (400) | PVP- I, Dettol, GLU | PRL, SAM, CAZ, CRO, FEP, CES, ATM, CT, NOR, AK, SXT, F |
| 25 | III (300) | PVP- I, GLU | PRL, SAM, CAZ, FEP, CES, NOR, F |
| 26 | -- | GLU | PRL, SAM, CAZ, CRO, FEP, CES, ATM, CT, SXT, F |
| 28 | -- | -- | PRL, SAM, CAZ, CRO, FEP, CES, ATM, NOR, OFX, GAT, AK, SXT, F |
| 29 | I (800) | -- | PRL, SAM, CAZ, CRO, CES, ATM, NOR, SXT |
| 30 | I (800) & III (150) | Dettol | PRL, SAM, CAZ, CRO, FEP, CES, ATM, CT, NOR, OFX, GAT, SXT, F |
| 31 | -- | Dettol | PRL, SAM, CAZ, CRO, FEP, CES, ATM, CT, NOR, OFX, GAT, SXT, F |
| 32 | -- | -- | PRL, SAM, CAZ, CRO, CES, ATM, NOR, AK, SXT |
| 33 | -- | Dettol | SAM, CAZ, NOR, AK, SXT, F |
| 34 | -- | Dettol | PRL, SAM, CAZ, CRO, FEP, CES, ATM, NOR, OFX, GAT, AK, SXT, F |
| 35 | I (1000) & III (150) | PVP- I, Dettol, GLU | PRL, SAM, CAZ, CRO, FEP, CES, ATM, CT, NOR, OFX, GAT, AK, SXT, F |
| 37 | I (800) & III (300) | PVP- I, Dettol, GLU | PRL, SAM, CAZ, FEP, CES, OFX, GAT, SXT |
| 38 | I (1500) | Dettol, GLU | PRL, SAM, CAZ, CRO, CES, ATM, NOR, AK, SXT, F |
| 39 | I (800) | PVP- I | PRL, SAM, CAZ, CRO, FEP, CES, ATM, NOR, OFX, GAT, SXT, F |
| 41 | -- | PVP- I | PRL, SAM, CAZ, CRO, FEP, CES, ATM, AK, SXT |
| 42 | III (300) | Dettol, GLU | PRL, SAM, CAZ, FEP, CES, OFX, GAT, SXT |
| 44 | I (250) & III (400) | PVP- I | SAM, CAZ, CRO, CES, SXT, F |
| 45 | -- | Dettol | PRL, SAM, CAZ, CRO, CES, ATM, NOR, SXT, F |
| 46 | -- | PVP- I | PRL, SAM, CAZ, CRO, FEP, CES, ATM, AK, SXT |
| 48 | I (1500) & III (300) | -- | PRL, SAM, CAZ, CRO, FEP, CES, ATM, NOR, SXT, F |
| 50 | -- | Dettol | PRL, SAM, CAZ, CRO, CES, ATM, NOR, AK, SXT |
| 51 | -- | Dettol | SAM, CAZ, NOR, AK, SXT, F |
| 54 | I (1500) & III (300) | -- | PRL, SAM, CAZ, CRO, FEP, CES, ATM, NOR, SXT, F |
| 55 | -- | PVP- I, GLU | PRL, SAM, CAZ, CRO, FEP, CES, ATM, NOR, OFX, GAT, AK, SXT, F |
| 56 | I (800) | -- | PRL, SAM ,CAZ, CRO, CES, ATM, NOR, SXT |
| 60 | III (300) | -- | PRL, SAM, CAZ, FEP, CES, NOR, F |
| 62 | I (250) & III (400) | Dettol | SAM, CAZ, CRO, CES, SXT, F |
| 64 | -- | -- | PRL, SAM, CAZ, CRO, CES, ATM, NOR, SXT |
| 66 | I (1500) | Dettol, GLU | PRL, SAM, CAZ, CRO, FEP, CES, IPM, ATM, NOR, AK, SXT, F |
| 67 | III (300) | Dettol, GLU | SAM, CAZ, FEP, NOR, OFX, GAT, AK, SXT, F |

**Table 2.** Continuing...

| | | | |
|---|---|---|---|
| 68 | -- | Dettol | PRL, SAM, CAZ, CRO, FEP, CES, ATM, NOR, OFX, GAT, AK, SXT, F |
| 69 | I (250) & III (400) | PVP- I, Dettol | SAM, CAZ, CRO, CES, SXT, F |
| 71 | -- | Dettol | SAM, CAZ, NOR, AK, SXT, F |
| 74 | I (800) | -- | PRL, SAM, CAZ, CRO, CES, ATM, NOR, SXT |
| 75 | -- | -- | PRL, SAM, CAZ, CRO, FEP, CES, ATM, AK, SXT |
| 76 | III (300) | GLU | PRL, SAM, CAZ ,FEP, CES, NOR, F |
| 78 | -- | PVP- I GLU | PRL, SAM, CAZ, CRO, FEP, CES, ATM, NOR, OFX, GAT, AK, SXT, F |
| 79 | -- | -- | PRL, SAM, CAZ ,CRO, FEP, CES,ATM, AK, SXT |
| 81 | I (250) & III (500) | Dettol | PRL, SAM, CAZ, CRO, FEP, CES, ATM, NOR, OFX, GAT, AK, SXT, F |
| 82 | -- | Dettol | SAM, CAZ, NOR, AK, SXT, F |
| 85 | -- | Dettol, GLU | SAM, CAZ, CRO, FEP, CES, NOR, AK, SXT, F |

sistance genes, as well as reduced susceptibility to biocides and antibiotic resistance profiles are shown in Table 2. The data showed that almost all of the integron I-positive isolates had reduced susceptibility to the tested biocides.

The correlation between the presence or absence of integron I/integron III and the susceptibility of the tested *K. pneumoniae* isolates to different antimicrobial agents was determined. Except for CRO, CES and SXT and Integron I, our results showed that no correlation was found between the presence of either integron I or integron III and the increase in resistance against most of the tested antimicrobial agents as shown in Table 3 and Fig. 4.

**Integron sequence analysis.** The variable and three conserved regions of the integrase gene were sequenced following PCR amplification of integron I. Multiple sequence alignment revealed that the 1.5 kb integron I have sequence similarity to *aadA* gene variants (*aadA1*) that encodes resistance genes against streptomycin and other aminoglycosides members (Table 4). The sequence alignment of the 2 kb integron I (isolate 24) showed the presence of N-acetyltrans-

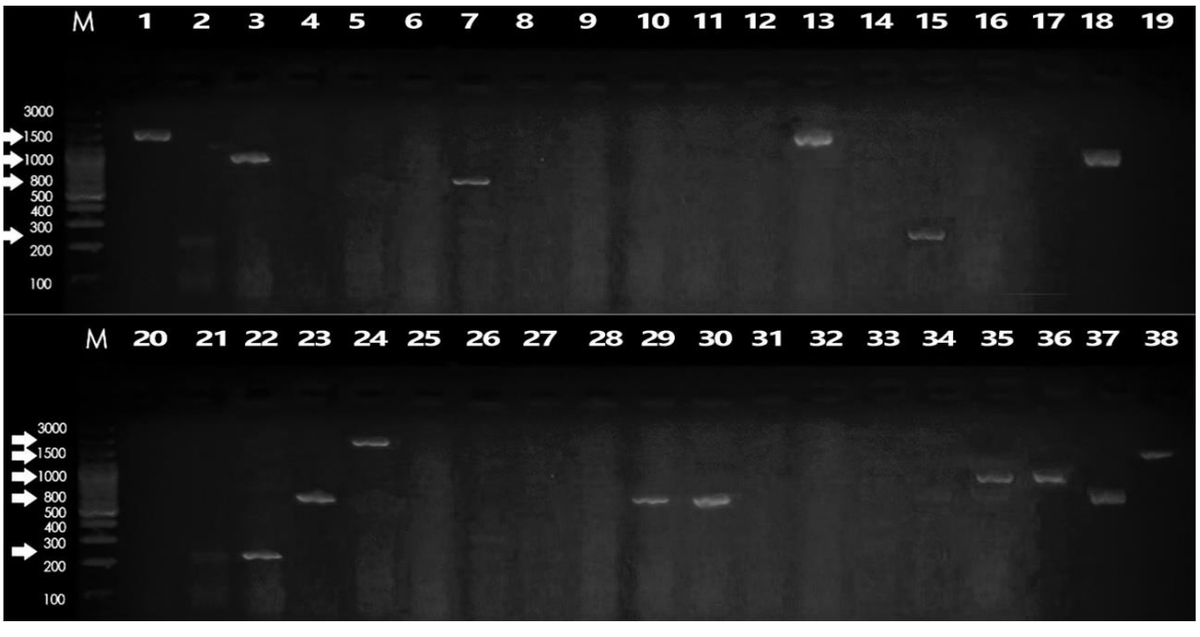



**Fig. 3.** Electrophoretic graph of conventional PCR products on 1.5% agarose gel stained with ethidium bromide of some representative *K. pneumoniae* isolates for the detection of integron I. Lanes M: represent 100 bp DNA ladder, Lane 18: positive control (reagent control mixture with DNA of the standard strain), Lane 19: negative control (reagent control mixture without DNA), Lanes 1-17 and 20-38: clinical *K. pneumoniae* isolates.

**Table 3.** Incidence of integrons and resistance percentage of *K. pneumoniae* isolates against tested antimicrobial agents.

| | Intergron I | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | + | | | | | | | | | | | | - | | | | | | | |
| | PRL | | SAM | | CAZ | | CRO | | CES | | AK | | SXT | | PRL | | SAM | | CAZ | | CRO | | CES | | AK | | SXT | |
| | R | S | R | S | R | S | R | S | R | S | R | S | R | S | R | S | R | S | R | S | R | S | R | S | R | S | R | S |
| Number | 21 | 4 | 25 | - | 25 | | 24 | 1 | 25 | - | 8 | 17 | 25 | - | 39 | 22 | 61 | | 58 | 3 | 36 | 25 | 41 | 20 | 24 | 37 | 39 | 22 |
| % | 84 | 16 | 100 | - | 100 | - | 96 | 4 | 100 | - | 32 | 68 | 100 | - | 64 | 36 | 100 | - | 95 | 5 | 59 | 41 | 67 | 33 | 39 | 61 | 64 | 36 |

| | Intergron III | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | + | | | | | | | | | | | | - | | | | | | | |
| | PRL | | SAM | | CAZ | | CRO | | CES | | AK | | SXT | | PRL | | SAM | | CAZ | | CRO | | CES | | AK | | SXT | |
| | R | S | R | S | R | S | R | S | R | S | R | S | R | S | R | S | R | S | R | S | R | S | R | S | R | S | R | S |
| Number | 14 | 7 | 21 | - | 21 | - | 12 | 9 | 18 | 3 | 6 | 15 | 17 | 4 | 46 | 19 | 65 | - | 62 | 3 | 48 | 17 | 48 | 17 | 26 | 39 | 47 | 18 |
| % | 67 | 33 | 100 | - | 100 | - | 57 | 43 | 86 | 14 | 29 | 71 | 81 | 19 | 71 | 29 | 100 | - | 95 | 5 | 74 | 26 | 74 | 26 | 40 | 60 | 72 | 28 |

*R: mean resistant, S: mean sensitive, +: mean present, -: mean absent

ferase AAC (6')-Il that is associated with aminoglycoside resistance. Moreover, this integron was found to harbor an additional gene of OXA β-lactamases that confers resistance to different types of penicillins. On the other hand, sequence alignment for integron I of 1 kb or smaller was found to encode resistance genes for trimethoprim as shown in Table 4.

## DISCUSSION

*Klebsiella pneumoniae* is a key pathogen implicated in community-acquired and nosocomial neonatal infections, with case fatality rates ranging from 18 to 68% (22, 23). In the recent years, the importance of the *K. pneumoniae* has grown due to the severe consequences of having limited antibiotic options, increased hospital costs, and poor neonatal outcomes (24).

Among the 86 *K. pneumoniae* isolates from neonates in this study, 66.27% were MDR. This detected resistance rate is higher than that reported before by Albasha et al. in Sudan (25), where the prevalence of MDR in *K. pneumoniae* isolates was only 35%. Thankfully, our clinical isolates are still highly sensitive to imipenem (97.7%) and colistin (91.9%) as reported previously by Albasha et al. in Sudan (25), and Jahanbin et al. in Iran (26). We found that the prevalence of resistance to β-lactams was the highest among the tested antibacterial agents, where resistance rates to ampicillin/sulbactam, ceftazidime, and cefoperazone/sulbactam were 98.8%, 88.4%, and 66.3%, respectively. These high β-lactam resistance rates were reported also in previous studies in Iran by Firoozeh et al. (2), and in Egypt by Hassuna et al. (27), which necessitates the future use of other alternatives. In the current study, resistance rates to ceftazidime, nitrofurantoin (the most important antimicrobial agents against *K. pneumoniae*), piperacillin (the most effective against *Enterobacteriaceae* and *Pseudomonas aeruginosa*) were 88.4%, 33.7%, and 69.8%, respectively. Similarly, resistance rates reported in previous studies in Sudan by Albasha et al. (25) and in Iran by Jahanbin et al. (26), and Heidary et al. (28), were 70%, 22.4%, and 62%, respectively. On the other hand, we found that the susceptibility rate of *K. pneumoniae* isolates toward the folate synthesis inhibitor Sulfamethoxazole/trimethoprim was less than that reported by Firoozeh et al. (2). Moreover, our study found a high resistance to aztreonam, which is in line with another study carried out by Heidary et al. (28) in Iran, who revealed a resistance rate of 64% among *K. pneumoniae* isolates. Similar to another study in Iran (28), we found that amikacin resistance rate of *K. pneumoniae* isolates was 34.9%.

Unfortunately, uncontrolled use of these antibacterial agents among people worldwide is highly common, which enhances the selective pressure on bacteria that leads to emergence of resistance. Our data revealed that imipenem, followed by colistin, and then ofloxacin were the best therapeutic options for treating *K. pneumoniae* infections.

Integrons have been found to be a major source of resistance genes and are thought to act as antimi-

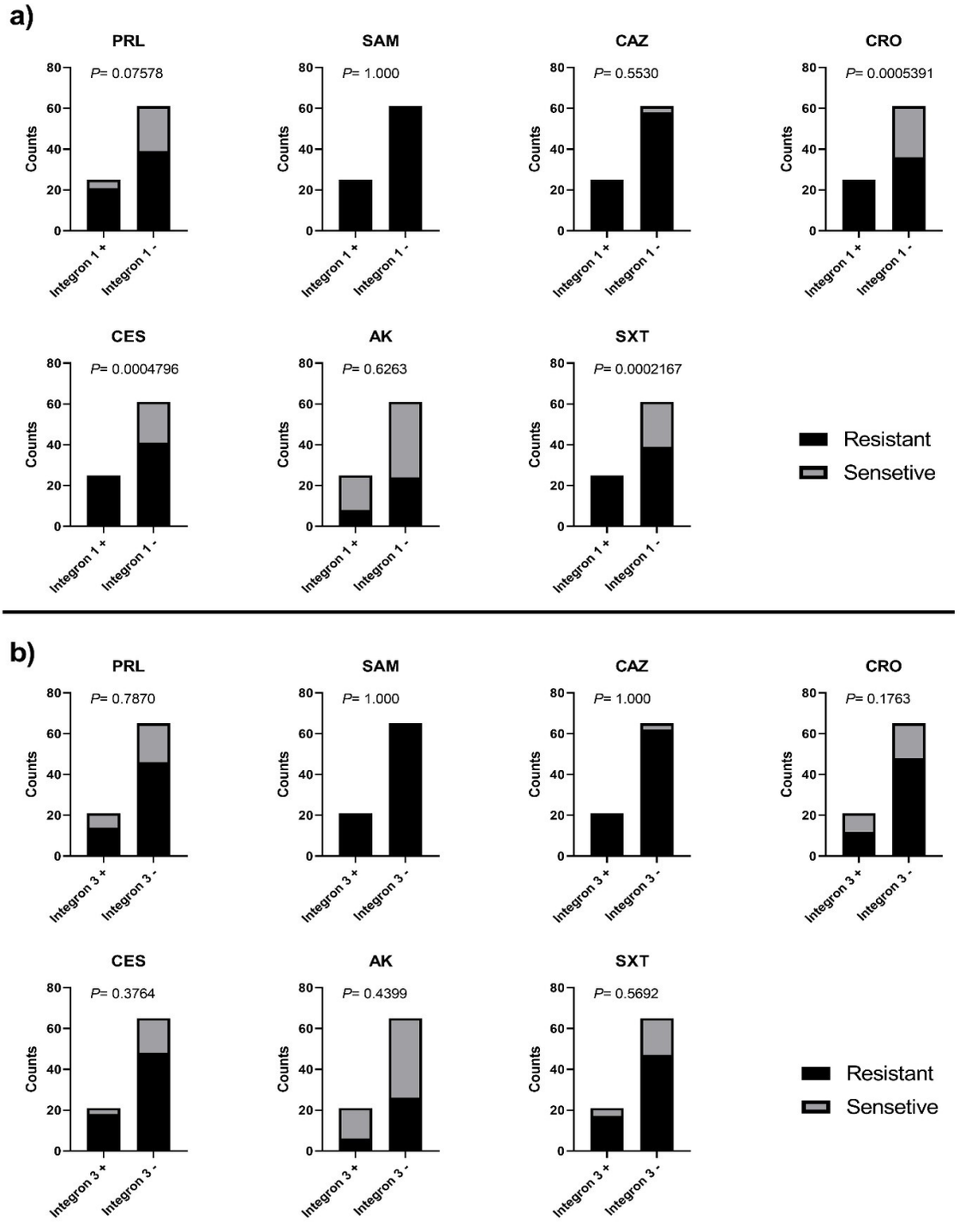



**Fig. 4.** Correlation between the susceptibility of the tested *K. pneumoniae* isolates to different antimicrobials agents and the presence or absence of: (a) integron I and (b) integron III

crobial resistance gene reservoirs in microbial communities including *K. pneumoniae* (29) that can be distributed among bacteria through horizontal gene transfer. The spread of integron-positive isolates in hospitals led to the spread of multi-drug resistant isolates (2). The current study reported the presence of class I integron in 40.3% of the *K. pneumoniae* isolates under investigation, which is relatively higher than that detected in previous studies carried out where the prevalence of integron I was 25.8% and

**Table 4.** Sequencing analysis for the selected *K. pneumoniae* isolates

| Isolates No | Sequence ID | CDS | Function |
|---|---|---|---|
| 1 | AM937245.2 | streptomycin / spectinomycin adenyltransferase | streptomycin / spectinomycin resistance |
| | CP048298.1 | ANT(3")-Ia family aminoglycoside nucleotidyltransferase AadA1 | aminoglycoside resistance protein; |
| 3 | FJ001873.1 | dihydrofolate reductase type VII | confers resistance to trimethoprim |
| | MK816931.1 | dihydrofolate reductase DfrA7 | resistance to trimethoprim |
| 7 | HQ880274.1 | dihydrofolate reductase | confers resistance to trimethoprim |
| | EU523055.1 | dihydrofolate reductase | confers resistance to trimethoprim |
| 13 | CP049307.1 | ANT(3")-Ia family aminoglycoside nucleotidyltransferase AadA1 | aminoglycoside resistance protein |
| | CP038443.1 | ANT(3")-Ia family aminoglycoside nucleotidyltransferase AadA1 | aminoglycoside resistance |
| 15 | MN543585.1 | Aminoglycoside 3"-nucleotidyltransferase | aminoglycoside resistance |
| 24 | CP034436.1 | aminoglycoside N-acetyltransferase AAC(6')-Il | aminoglycoside resistance |
| | CP058133.1 | oxacillin-hydrolyzing class D beta-lactamase OXA-10 | Penicillin-binding protein transpeptidase domain |

12.2% (20, 30). On the other hand, another study carried out in China by Xu et al. (31) showed a relatively higher percentage of class I integron (60.1%) among *K. pneumoniae* isolated from Chinese tertiary hospitals.

Integron III was found only in 20 of the MDR isolates (35%). This is higher than a previous study carried out in the Netherlands, which discovered that only 10.97% of the tested isolates had integron III (32). On the other hand, studies conducted in Iran by Firoozeh et al. (2) and Jahanbin et al. (26), reported that none of the studied MDR *K. pneumoniae* isolates had class III integrons. This observed increased prevalence of integron III among our MDR *K. pneumoniae* isolates represents an additional player that might contribute significantly in the spread of bacterial resistance.

Our study reports the absence of class II integron among the tested *K. pneumoniae* isolates which was similar to findings obtained by Xu et al. in China (31), Zeighami et al. (33), and Mobarak-Qamsari in Iran (34).

Sequencing of class I integron revealed that aminoglycosides and folate synthesis inhibitors gene cassettes were predominant. Likewise, Liao et al. (35) and Xu et al. (31) reported the abundance of trimethoprim (*dfr*) and aminoglycosides (*aac, aad*) in class I integrons. On the other hand, other resistance genes may be found, however, at lower abundance. We detected the presence of oxacillin-hydrolyzing class D beta-lactamase OXA-10 in only one of the sequenced integrons. Poirel et al. showed that the acquired class D β-lactamase genes are mostly linked to class I integron or to insertion sequences (36). Correlation studies for the association between the presence of integrons and the observed antimicrobial resistance revealed that resistance to CRO, CEF and SXT, where the only statistically associated with integron I presence. Therefore, integron I in MDR *K. pneumoniae* seems to contribute only to limited antibacterial and biocide resistance.

On the other hand, our observations from susceptibility of integron positive and integron negative isolates of *K. pneumoniae* to different biocides and the absence of detected genes associated with biocide resistance in the sequenced integrons from isolates resistant to different biocides, suggest that there is no current linkage between the presence of integrons and resistance to the tested biocides. Moreover, our results indicate that there is no possible linkage between antibacterial resistance and biocide resistance mediated via integrons as this linkage has been a point of concern for several decades (37).

## CONCLUSION

The presence of integron I in MDR *K. pneumoniae* isolates from neonatal sepsis may contribute only to limited antibacterial and biocide resistance, however,

it does not seem to be the major contributor. Thankfully, imipenem, colistin and ofloxacin are still valid options for treatment, however, to preserve their activity implementation of antimicrobial stewardship and antibiotic policies becomes a must.

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