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Sub-Inhibitory N-Acetylcysteine and L-Glutamine Partially Restore Ceftriaxone Susceptibility in Multidrug-Resistant *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*

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ABSTRACT

Background: *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are Gram-negative pathogens commonly recovered from hospital surfaces, where multidrug resistance (MDR) restricts treatment options. N-acetylcysteine (NAC) and L-glutamine have each been proposed as non-antibiotic adjuvants capable of restoring antibiotic activity against resistant bacteria.
Methods: A total of 50 surface swabs were collected from medical-device surfaces in a Sebha hospital; 18 yielded Gram-negative isolates identified as *K. pneumoniae* (n = 12) or *P. aeruginosa* (n = 6). These isolates were tested by Kirby-Bauer disk diffusion against six antibiotics before and after 4-hour exposure to sub-inhibitory (0.5 mg/mL) NAC or L-glutamine, with each isolate serving as its own control. Inhibition zones were interpreted using CLSI 2024 breakpoints; paired comparisons used the Wilcoxon signed-rank test and Fisher's exact test. **Results:** Baseline resistance was 100% for ampicillin, cefoxitin and ertapenem, 89% for ceftriaxone and ceftazidime, and 11% for co-trimoxazole. Mean ceftriaxone zone diameter increased from 11.8 mm to 18.0 mm after NAC exposure ($p = 0.005$) and to 17.6 mm after glutamine exposure ($p = 0.009$), converting 6 of 18 isolates from resistant to susceptible under each adjuvant ($p = 0.018$ and $p = 0.041$, respectively). Neither adjuvant produced a significant change in susceptibility to ampicillin, cefoxitin, ertapenem, ceftazidime or co-trimoxazole; a single ampicillin conversion was observed, after glutamine exposure only. **Conclusion:** Sub-inhibitory NAC and L-glutamine each partially restored ceftriaxone susceptibility in these environmental isolates without a comparable effect on the other antibiotics tested. The findings point to a narrow, ceftriaxone-specific resensitization rather than a broad antimicrobial-potentiating action for either compound.

التركيزات دون المثبطة من N-Acetylcysteine و L-Glutamine تستعيد جزئياً الحساسية تجاه Ceftriaxone في بكتيريا *Klebsiella pneumoniae* و*Pseudomonas aeruginosa* المقاومة للأدوية المتعددة

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الكلمات المفتاحية:
مساعدات المضادات الحيوية
سيفترياكسون
الأسطح البيئية في المستشفيات
كلبسيلا الرئوية
إل-جلوتامين

الملخص

الخلفية (Background): تُعد بكتيريا *Klebsiella pneumoniae* و*Pseudomonas aeruginosa* من المرضات سالبة الجرام التي تُعزل عادةً من أسطح المستشفيات، حيث تحد المقاومة للأدوية المتعددة (MDR) من الخيارات العلاجية المتاحة. وقد اقتُرح استخدام كل من N-acetylcysteine (NAC) و L-glutamine كعوامل مؤازرة (مساعدة) غير حيوية، قادرة على استعادة فعالية المضادات الحيوية ضد البكتيريا المقاومة. المنهجية (Methods): جُمعت 50 مسحة سطحية من أسطح الأجهزة الطبية في أحد مستشفيات مدينة سبها؛ وأسفرت 18 مسحة منها عن عزلات سالبة الجرام حُددت على أنها *K. pneumoniae* (العدد = 12) أو *P. aeruginosa* (العدد = 6). اختُبرت هذه العزلات باستخدام طريقة انتشار الأقراص (Kirby-Bauer) ضد ستة مضادات حيوية

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قبل وبعد التعرض لمدة 4 ساعات لتركيزات دون المثبطة (0.5 مجم/مل) من NAC أوL-glutamine ، بحيث أُعتبرت كل عزلة بمثابة عينة ضابطة لنفسها. فُسرت مناطق التثبيط باستخدام نقاط الحد المعتمدة من معهد المعايير السريرية والمخبرية (CLSI) لعام 2024؛ وأُجريت المقارنات المزدوجة باستخدام اختبار ويلكوكسون ذي الرتب المؤشرة (Wilcoxon signed-rank test) واختبار فيشر الدقيق (Fisher's exact test). النتائج (Results): بلغت نسبة المقاومة الأساسية 100% لكل من ampicillin و cefoxitin وertapenem ، و89% لـ ceftriaxone وceftazidime ، و11% لـ co-trimoxazole. ازداد متوسط قطر منطقة التثبيط لـ ceftriaxone من 11.8 ملم إلى 18.0 ملم بعد التعرض لـ NAC القيمة الاحتمالية (p = 0.005) وإلى 17.6 ملم بعد التعرض لـ L-glutamine (القيمة الاحتمالية p = 0.009) ، مما أدى إلى تحويل 6 عزلات من أصل 18 من حالة "مقاومة" إلى "حساسة" تحت تأثير كل عامل مؤازر (p = 0.018 و p = 0.041) ، على التوالي. (ولم يُحدث أي من العاملين المؤازرين تغيراً ذا دلالة إحصائية في الحساسية تجاهampicillin ، أوcefoxitin ، أوertapenem ، أوceftazidime ، أو-co trimoxazole؛ في حين لُوحظت حالة تحول وحيدة نحو الحساسية لـ ampicillin بعد التعرض لـ L-glutamine فقط. الخلاصة :(Conclusion) أسهم التعرض للتركيزات دون المثبطة من NAC و L-glutamine في الاستعادة الجزئية للحساسية تجاه ceftriaxone في هذه العزلات البيئية، دون إحداث تأثير مماثل على المضادات الحيوية الأخرى التي تم اختبارها. تشير هذه النتائج إلى تأثير ضيق النطاق ومحدد لإعادة التحسيس تجاهceftriaxone ، وليس تأثيراً مؤازراً وواسع النطاق لمضادات الميكروبات لأي من المركّبين.

Introduction

Klebsiella pneumoniae and *Pseudomonas aeruginosa* are Gram-negative bacteria frequently isolated from hospital surfaces and medical equipment, where they persist for extended periods and act as reservoirs for healthcare-associated infection [1,2]; Kramer et al. showed that such organisms can survive on dry hospital surfaces for weeks to months [3], and environmental surveys have since recovered multidrug-resistant (MDR) strains of both species from a substantial proportion of high-touch surfaces across hospital departments [4]. *K. pneumoniae*, a capsulated, lactose-fermenting bacillus, is a leading cause of catheter-associated urinary tract infection, ventilator-associated pneumonia and bloodstream infection, particularly among neonatal and immunocompromised patients [5], while *P. aeruginosa*, an aerobic non-fermenter adapted to moist environments such as sink traps and respiratory equipment, produces an array of virulence factors and is a frequent cause of respiratory and wound infection [6]; both organisms form biofilms readily, a property that keeps routine surface decontamination central to infection control even though the ideal cleaning protocol remains debated [7]. Resistance in these species commonly involves several mechanisms acting together, extended-spectrum β-lactamases and carbapenemases in *K. pneumoniae*, AmpC cephalosporinases and metallo-β-lactamases in *P. aeruginosa*, together with porin loss, efflux overexpression and biofilm-mediated protection that can raise antibiotic tolerance up to 1000-fold above planktonic levels [2,8], so that disabling a single mechanism is often not enough to restore antibiotic activity. Because developing new antibiotics is slow and costly, attention has turned to non-antibiotic adjuvants that modify bacterial susceptibility to drugs already in clinical use: N-acetylcysteine (NAC), a thiol-containing mucolytic agent, disrupts biofilm architecture by reducing the disulfide bonds that stabilize the extracellular matrix and has been shown to degrade established biofilms and inhibit new biofilm formation across several bacterial species [9], with subsequent work proposing its adjunctive use specifically against MDR Gram-negative infection [10], while L-glutamine acts through a different route, increasing bacterial membrane permeability and disrupting the transmembrane pH gradient in ways that enhance intracellular antibiotic accumulation and impair efflux [11,12]. Most of the evidence for this glutamine effect, however, comes from *Escherichia coli* and Gram-positive organisms such as methicillin-resistant *Staphylococcus aureus* rather than from the capsulated, biofilm-forming *Klebsiella* and *Pseudomonas* strains that dominate hospital environmental contamination, and comparable resistance and adjuvant-response data from Libyan hospitals have not previously been reported. The present study therefore characterized the baseline antibiotic susceptibility of *Klebsiella* and *Pseudomonas* isolates recovered from medical-device surfaces in a hospital in Sebha, Libya, and evaluated whether sub-inhibitory N-acetylcysteine or L-glutamine could alter their susceptibility to a panel of commonly used antibiotics.

2. Materials and Methods

2.1 Study design and setting

This laboratory-based study used a paired, before-and-after design in which each bacterial isolate served as its own control, so that susceptibility measured after adjuvant exposure could be compared directly against that same isolate's baseline. Sample processing, bacterial identification and susceptibility testing were carried out in the microbiology laboratory of the Faculty of Science, Sabha University; environmental samples were obtained from the Sebha Women's and Maternity Hospital.

2.2 Sample collection

A total of 50 surface swabs were collected from high-touch surfaces and medical equipment in critical hospital areas, including the neonatal intensive care unit and operating theatres. Target sites comprised infant incubators, mechanical ventilators, examination beds and instrument trolleys. Sterile cotton swabs pre-moistened with sterile physiological saline (0.85% NaCl) were used to sample an approximate 25 cm² area of each surface. Each swab was placed into a tube containing 5 mL nutrient broth (Oxoid Ltd., Basingstoke, UK) as transport medium and delivered to the laboratory under refrigeration (approximately 4°C) within two hours of collection.

2.3 Isolation and identification

In the laboratory, transport tubes were vortexed to release adherent bacteria, and 100 µL of each suspension was plated onto MacConkey agar (Oxoid Ltd., UK), to recover Gram-negative bacteria and distinguish lactose-fermenting from non-fermenting colonies, and onto a chromogenic agar (HiMedia Laboratories, India) for preliminary visual differentiation by colony colour. Plates were incubated aerobically at 37°C for 24-48 h. Presumptive colonies were purified by sub-culture on nutrient agar (Oxoid Ltd., UK) before identification. Of the 50 swabs processed, 18 yielded Gram-negative bacterial growth that was subsequently confirmed as *Klebsiella* or *Pseudomonas* by the biochemical and identification methods described below; the remaining swabs showed no growth, mixed non-target flora, or organisms outside the scope of this study and were not carried forward.

Lactose-fermenting, mucoid, pink colonies suspected to be *Klebsiella* were identified using a conventional biochemical battery comprising the indole, methyl red, Voges-Proskauer and citrate-utilization tests (the IMViC panel), together with triple sugar iron (TSI) agar, urease production and a motility test (media supplied by Oxoid Ltd., UK, and HiMedia Laboratories, India), alongside the API 20E miniaturized

identification system (bioMérieux, Marcy-l'Étoile, France), used according to the manufacturer's instructions and interpreted with the accompanying analytical profile index. *K. pneumoniae* was distinguished from other Enterobacterales by a positive Voges-Proskauer reaction, negative indole and motility, urease positivity, and typical TSI fermentation, consistent with standard diagnostic criteria for the genus. Lactose-non-fermenting, oxidase-positive colonies suspected to be *P. aeruginosa* were confirmed by the oxidation-fermentation (Hugh-Leifson) test, growth on cetrimide agar (Oxoid Ltd., UK), and pigment production (pyocyanin and pyoverdine) assessed under normal and long-wave ultraviolet light. Confirmed isolates were preserved on nutrient agar slants at 4°C for short-term use and in nutrient broth containing 20% glycerol at −20°C for long-term storage.

2.4 Antibiotic susceptibility testing

Susceptibility was assessed by the Kirby-Bauer disk diffusion method following CLSI 2024 (M100, 34th edition) guidance [13]. A saline suspension of each isolate was adjusted to a 0.5 McFarland standard (~1.5×10^8 CFU/mL) and inoculated onto Mueller-Hinton agar (Oxoid Ltd., Basingstoke, UK) as a confluent lawn. Six antibiotic disks (Oxoid Ltd., UK) representing distinct classes were applied (Table 1). After incubation at 37°C for 16-18 h, inhibition-zone diameters were measured in millimetres with a calibrated ruler and isolates were categorized as susceptible (S), intermediate (I) or resistant (R) using CLSI 2024 zone-diameter breakpoints, applying the *Pseudomonas*-specific ceftazidime breakpoints to *Pseudomonas* isolates and accounting for the antibiotics to which *P. aeruginosa* is intrinsically resistant or for which no validated disk breakpoint exists.

2.5 Preparation and application of the adjuvants

N-acetylcysteine and L-glutamine (both Sigma-Aldrich, St. Louis, MO, USA; analytical/reagent grade) were each weighed on an analytical balance and dissolved in sterile physiological saline to prepare a stock solution, then diluted in the same diluent to a final working concentration of 0.5 mg/mL. This concentration was selected as sub-inhibitory based on prior reports that it does not itself suppress bacterial growth but can modulate biofilm-related physiology [14]. Both solutions were adjusted to pH 7.0 with sterile 0.1 N NaOH or HCl as required and sterilized by passage through a 0.22 µm membrane filter rather than by autoclaving, to avoid heat-related degradation of the thiol and amino-acid groups. For each isolate, two parallel bacterial suspensions were prepared in Mueller-Hinton broth (Oxoid Ltd., UK), one supplemented with 0.5 mg/mL NAC and the other with 0.5 mg/mL L-glutamine and incubated at 37°C for 4 h to allow interaction between the adjuvant and the bacterial cell envelope. Following incubation, each suspension was re-standardized to a 0.5 McFarland turbidity standard and used immediately for disk-diffusion testing as described in Section 2.4, so that the post-exposure inhibition zones could be compared directly with the same isolate's own baseline measurement.

2.6 Statistical analysis

Inhibition-zone diameters were treated as paired data. Because zone diameters were not normally distributed and several antibiotics showed floor effects (many zero values), baseline-versus-adjuvant comparisons used the Wilcoxon signed-rank test. Categorical shifts in interpretation, most importantly resistant to susceptible, were assessed with Fisher's exact test on 2 × 2 tables of susceptible versus non-susceptible counts. Statistical significance was set at $p < 0.05$. All analyses were performed on the complete raw zone-diameter dataset, and susceptibility categories were assigned directly from CLSI 2024 breakpoints applied to the measured diameters, the statical analysis was done using Minitab 19

3. Results

3.1 Baseline antibiotic susceptibility

Of 50 surface swabs collected, 18 yielded Gram-negative bacterial isolates: 12 were identified as *K. pneumoniae* and 6 as *P. aeruginosa*. Baseline susceptibility results for these 18 isolates are summarized in Table 2. Resistance was 100% for ampicillin, cefoxitin and ertapenem (18/18 isolates each). Resistance to ceftriaxone and ceftazidime was 89% (16/18 isolates each) (Figure 1), with 1 isolate intermediate and 1 susceptible to each agent. Resistance to co-trimoxazole was 11% (2/18 isolates), with 6 isolates intermediate and 10 susceptible.

Table 1. Baseline antibiotic susceptibility of the 18 environmental isolates, interpreted by CLSI 2024 disk-diffusion breakpoints.

Antibiotic	Resistant (R)	Intermediate (I)	Susceptible (S)	Resistance (%)
Ampicillin (AMP)	18	0	0	100%
Cefoxitin (CX)	18	0	0	100%
Ertapenem (ETP)	18	0	0	100%
Ceftriaxone (CRO)	16	1	1	89%
Ceftazidime (CAZ)	16	1	1	89%
Co-trimoxazole (COT)	2	6	10	11%

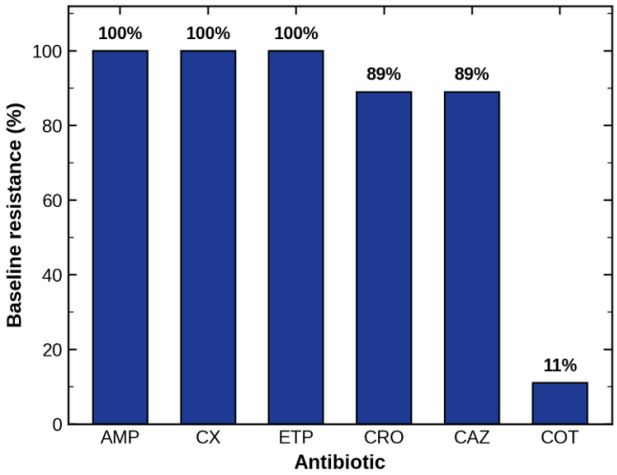


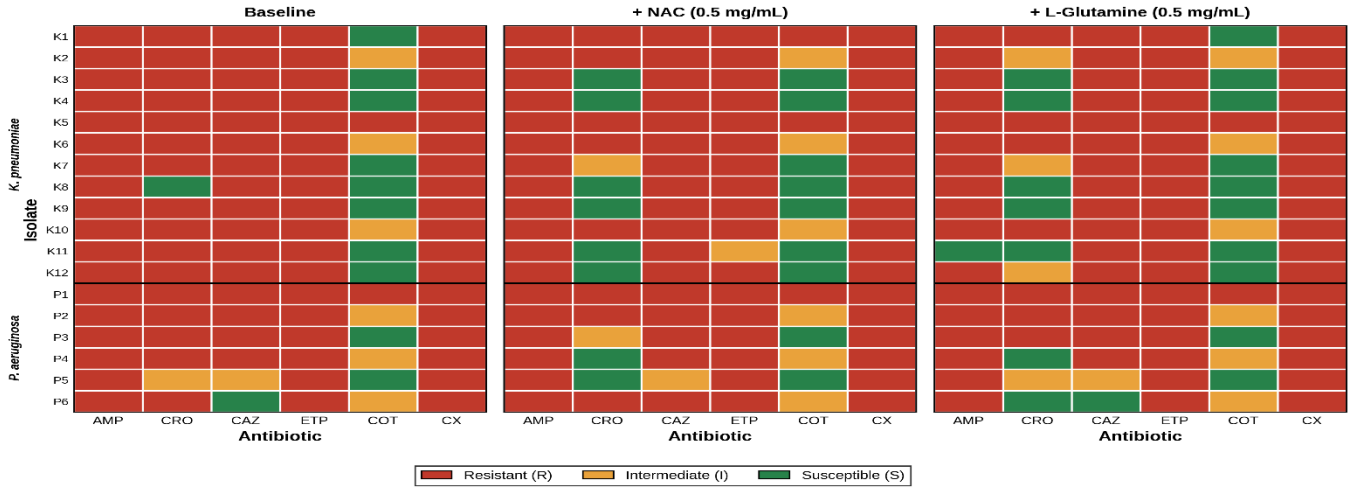
Figure 1. Baseline resistance among the 18 environmental isolates, ordered by resistance percentage. Ampicillin, cefoxitin and ertapenem were resisted by every isolate.

3.2 Change in inhibition-zone diameter after adjuvant exposure

Mean inhibition-zone diameters before and after adjuvant exposure are summarized in Table 3. The mean ceftriaxone zone diameter increased from 11.8 ± 6.1 mm at baseline to 18.0 ± 9.0 mm after NAC exposure (Wilcoxon signed-rank test, $p = 0.005$) and to 17.6 ± 9.4 mm after glutamine exposure ($p = 0.009$). Among the 12 *K. pneumoniae* isolates alone, the mean ceftriaxone zone rose from 12.2 mm at baseline to 19.6 mm after NAC exposure and 19.1 mm after glutamine exposure(Figure 2). Zone-diameter changes for ampicillin, cefoxitin, ertapenem, ceftazidime and co-trimoxazole were not statistically significant for either adjuvant (Table 2).

Table 2. Mean ± SD inhibition-zone diameter (mm) at baseline and after 4-hour exposure to NAC or L-glutamine, all 18 isolates. P-values are for the paired Wilcoxon signed-rank test comparing each adjuvant condition with baseline.

Antibiotic	Baseline (mm)	+ NAC (mm)	p (NAC)	+ Glutamine (mm)	p (Glutamine)
Ampicillin	1.1 ± 3.2	1.3 ± 3.6	1.000	3.1 ± 6.0	0.225
Ceftriaxone	11.8 ± 6.1	18.0 ± 9.0	0.005	17.6 ± 9.4	0.009
Ceftazidime	2.5 ± 7.5	0.8 ± 3.4	0.317	2.2 ± 6.5	0.735
Ertapenem	0.9 ± 3.7	1.1 ± 4.6	1.000	0.0 ± 0.0	0.317
Co-trimoxazole	15.4 ± 4.3	14.4 ± 5.5	0.454	15.8 ± 4.8	0.657
Cefoxitin	0.0 ± 0.0	0.0 ± 0.0	-	0.0 ± 0.0	-



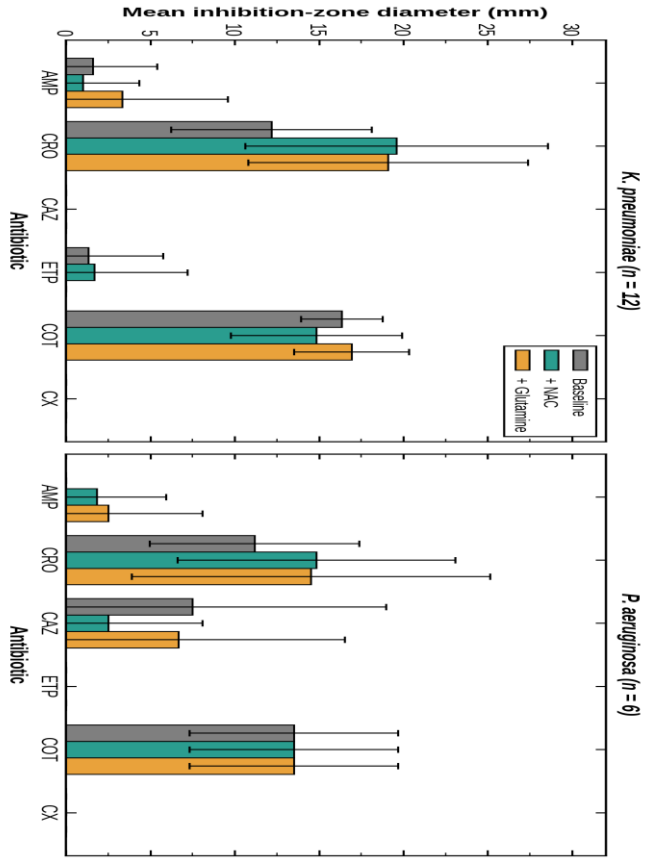


Figure 2. Mean inhibition-zone diameter (± SD) for each antibiotic before treatment (baseline) and after exposure to NAC or L-glutamine, shown separately for K. pneumoniae (n = 12) and P. aeruginosa (n = 6).

3.3 Categorical resistant-to-susceptible conversions

Six of 18 isolates changed classification from resistant to susceptible for ceftriaxone after NAC exposure (Fisher's exact test, $p = 0.018$), and 6 of 18 isolates converted after glutamine exposure ($p = 0.041$) (Figure 3). For ampicillin, no conversions occurred after NAC exposure; one isolate converted from resistant to susceptible after glutamine exposure ($p = 1.000$). No conversions occurred for cefoxitin, ertapenem, ceftazidime or co-trimoxazole under either adjuvant (Table 4). Two isolates showed a reversion from susceptible or intermediate to resistant after NAC exposure: one for co-trimoxazole and one for ceftazidime. Two isolates, one *K. pneumoniae* and one *P. aeruginosa*, were resistant to all six antibiotics at baseline and remained resistant to all six after exposure to either adjuvant.

Table 3. Number of resistant-to-susceptible conversions per antibiotic after NAC or L-glutamine exposure (of 18 isolates), with Fisher's exact test p-values for the categorical shift.

Antibiotic	Conversions, NAC	p (NAC)	Conversions, Glutamine	p (Glutamine)
Ampicillin	0	1.000	1	1.000
Ceftriaxone	6	0.018	6	0.041
Ceftazidime	0	1.000	0	1.000
Ertapenem	0	-	0	-
Co-trimoxazole	0	1.000	0	1.000
Cefoxitin	0	-	0	-

Figure 3. Susceptibility category (red = resistant, amber = intermediate, green = susceptible) for all 18 isolates against six antibiotics under three conditions: baseline, after NAC, and after L-glutamine. The horizontal line separates K. pneumoniae (top 12 rows) from P. aeruginosa (bottom 6 rows)

4. Discussion

This study set out to characterize the baseline antibiotic susceptibility of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* isolates recovered from hospital surfaces in Sebha, Libya, and to determine whether sub-inhibitory exposure to N-acetylcysteine or L-glutamine could alter their susceptibility to a panel of commonly used antibiotics. The isolates examined here were highly resistant at baseline, with universal resistance to ampicillin, cefoxitin and ertapenem and resistance above 85% for ceftriaxone and ceftazidime; only co-trimoxazole retained meaningful activity, and even then in a minority of isolates. By CLSI criteria this places most isolates in the multidrug-resistant category, and the complete loss of ertapenem activity, a carbapenem generally reserved for difficult infections, indicates that some isolates approach an extensively drug-resistant phenotype. This resistance burden is consistent with the recognized role of hospital surfaces as reservoirs for multidrug-resistant Gram-negative bacteria [2,4], and it illustrates that ordinary environmental surfaces, not only clinical specimens, can harbor organisms with very limited remaining treatment options.

Exposure to sub-inhibitory NAC or L-glutamine produced a similar and fairly narrow effect: both adjuvants raised the mean ceftriaxone inhibition zone by six to seven millimetres and converted six of eighteen isolates from resistant to susceptible, while producing no significant change in susceptibility to the other five antibiotics tested. That two adjuvants with different proposed mechanisms, NAC acting on the biofilm matrix [9,10] and glutamine acting on membrane permeability [11,12], converged on the same antibiotic suggests that ceftriaxone resistance in this collection was limited mainly by drug access rather than by irreversible enzymatic inactivation. Resistance to cefoxitin and ertapenem behaved differently: it remained unchanged under either adjuvant, a pattern more consistent with inactivation by dedicated hydrolytic enzymes, AmpC-type cephalosporinases for cefoxitin and carbapenemases for ertapenem, that destroy the drug regardless of how efficiently it reaches the periplasm [2]. Read together, these results point to specific, access-limited resistance phenotypes, rather than multidrug resistance in general, as the setting in which these adjuvants are likely to be useful.

The response to ampicillin is worth addressing on its own, since it diverges from what the published glutamine literature would predict. Glutamine's reported mechanism, increased membrane permeability

and disrupted proton-motive force, should in principle also improve activity against uptake-limited resistance such as ampicillin resistance [11,12]; here, only one of eighteen isolates converted, and the change in mean zone diameter was not statistically significant. Much of the evidence supporting glutamine's permeabilizing effect comes from *Escherichia coli* and Gram-positive organisms such as MRSA, neither of which shares the thick capsule or porin architecture of *Klebsiella* and *Pseudomonas*, and an adjuvant effect calibrated on those organisms may simply translate less well to a heavily capsulated Gram-negative envelope. Considered alongside the ceftriaxone result, this indicates that glutamine's resensitizing activity in these isolates was genuine but did not extend across every β-lactam tested.

Two isolates, one *K. pneumoniae* and one *P. aeruginosa*, remained resistant to all six antibiotics under every condition tested. This pattern reinforces the same interpretation offered above: where resistance is multiple and enzymatic, disrupting a single barrier, whether the biofilm matrix or membrane permeability, is unlikely on its own to restore antibiotic activity. Taken as a whole, the findings support a specific rather than general role for these adjuvants: they appear capable of recovering a defined, access-limited resistance phenotype rather than acting as broad resistance-reversing agents, which has practical implications for where such compounds might reasonably be tested next and where they would not be expected to help.

These findings should be read with several caveats. Identification relied on conventional biochemical testing and the API 20E system rather than molecular methods, and the specific resistance enzymes were not genotyped, so the enzymatic-versus-permeability explanation offered above is inferred from the susceptibility pattern rather than demonstrated directly. Susceptibility was assessed by a single disk-diffusion measurement per isolate per condition, exposure was tested at one concentration and one duration, and the collection was limited to eighteen isolates from a single hospital; the magnitude of the ceftriaxone effect, and its absence for the other agents tested, should be confirmed in a larger, genotyped isolate collection before the findings are generalized. The work is also entirely *in vitro*; whether the same resensitization would be observed, or would translate into improved outcomes, in an infected host remains to be tested.

5. Conclusion

Environmental *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* isolates recovered from hospital surfaces in Sebha, Libya, displayed a severe multidrug-resistant phenotype at baseline, including complete resistance to ertapenem. Sub-inhibitory N-acetylcysteine and L-glutamine each partially restored ceftriaxone susceptibility, converting six of eighteen isolates from resistant to susceptible, but neither adjuvant produced a comparable effect against ampicillin, cefoxitin, ertapenem, ceftazidime or co-trimoxazole. These results indicate that non-antibiotic adjuvants of this kind can recover a specific, access-limited cephalosporin resistance without acting as general resistance modifiers, and they provide baseline environmental resistance data for this region. Confirming the underlying resistance mechanisms by molecular methods, and testing the ceftriaxone-adjuvant effect in a larger isolate collection and across a range of exposure conditions, are appropriate next steps before any clinical application is considered.

Declarations

Ethics approval: Not applicable; the study involved environmental sampling of hospital surfaces and did not include human participants .

Consent for publication: Not applicable.

Availability of data: The raw inhibition-zone measurements underlying this analysis are available from the corresponding author on reasonable request.

Competing interests: The authors declare no competing interests.

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Authors' contributions: [Shamsi Saad : Conceptualization, , Investigation, Formal analysis, Writing - original draft, Writing - review & editing, Supervision S*alimah Aboqulqasim*, R*uqayah* M*ohammed*, Mariam Masoud : Methodology.]

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