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Combination agent antibiogram of common antimicrobial classes viz β-lactam drugs, aminoglycosides, fluoroquinolones and folate antagonist for major gram-negative pathogens—an innovative approach to assist appropriate empirical decisions

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ABSTRACT

Introduction: Prescribers have a tendency to empirically start on combination antimicrobial regimens instead of monotherapy in scenarios such as hemodynamically unstable or critically sick patients. However, most of these choices are based on their own experience and not evidence based. In our study, we have developed combination agent antibiogram for major gram-negative pathogens to assist appropriate empirical decisions.

Materials and Methods: During the period January-December, 2023, susceptibility data of blood culture isolates were collated to develop combination antibiogram using the standard CLSI M39 guideline for five major gram-negative pathogens—*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Acinetobacter baumannii* complex and Pseudomonas aeruginosa. The individual susceptibility profiles of the five gram-negative organisms and their aggregate was used generate antibiogram. The combination regimens chosen for developing combination antibiogram comprised of agents belonging to two different antimicrobial classes- β-lactams, quinolones, aminoglycosides or folate antagonist. The S% to combination agents was calculated as percentage of isolates susceptible to either of the antimicrobial agents.

Results: Many combination regimens were found to demonstrate statistically significant improvement in susceptibility (S)% compared to the that of individual antimicrobial agent for *E. coli* and gram-negative bacilli level, to some extent for *K. pneumoniae*, but such finding was not observed for *P. aeruginosa*, *A. baumannii* or E. cloacae.

Conclusion: Combination agent antibiogram for empirical choice is an excellent tool to provide evidence-based recommendations on selecting correct combination of antimicrobial agents as empirical choices. Every healthcare facility should prepare combination agent Antibiogram for the most commonly used antimicrobial combination regimens after discussion with the prescribers.

1. Introduction

In a routine antibiogram, the susceptibility data of an organism species are displayed as percentages (S%) of organisms susceptible to individual antimicrobial agents

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that are active for that species.[1] This data is useful when only one antimicrobial agent is chosen for empirical therapy. However, there are specific clinical scenarios where a combination of two different antimicrobial agents may be required to use as empirical therapy. This is more commonly used for gram-negative bacterial infections especially sepsis, compared to gram-positive bacterial infections. The indications for empirical therapy with double gram-negative coverage include immunocompromised patients, critically ill patients, patients with a past history of multi-drug resistant organisms (MDROs) infection, patients with haematological malignancies receiving chemotherapy clinically presented as febrile neutropenia, and certain types of infections such as hospital-acquired and ventilator-associated pneumonia, etc.[1,2]

In order to decide, which antimicrobial agent combinations are more effective, combination agent antibiograms are used, which analyses the S% data of isolates to various combinations of antimicrobial agents and subsequently compare the data with the S% to individual antimicrobial agents. It reports the S% of isolates to the select antimicrobial agent combinations, compared to traditional antibiograms, which report S% only for a single agent. These data can assist in developing specific protocols for empirical combination therapy for specific indications. A facility can create an institutional antimicrobial policy to use combination therapy with two agents from different antimicrobial classes as empirical therapy if the combined S% to either of the combination agents is found to be significantly higher than S% to individual antimicrobial agents.[3,4] Clinical and Laboratory Standards Institute (CLSI) provides M-39 document which provides the details on the preparation of combination agent antibiogram.[2] Even if various national & international standard guidelines exist of common syndromic empirical antimicrobial use on common infective syndrome, these guidelines don't elaborate anything on the use of combination agent antibiogram for making empirical therapeutic choices.[5,6]

In double antimicrobial coverage, the two antimicrobial agents used should differ in their mechanisms of action. The most frequent combination used by the clinicians for gram-negative infection include a β-lactam group of agents along with another agent of a different antimicrobial class, such as aminoglycosides or quinolones.[7,8] In our study, there is paucity of data in literature on combination agent antibiogram. Therefore, we have conducted this innovative study to construct a combination agent antibiogram for major gram-negative pathogens, comprising of various combinations of two agents—one from β-lactam group of agents and another agent from aminoglycosides or quinolones.

2. Materials and Methods

A large-scale teaching institution in South India, undertook this observational prospective study from January to December 2023 in its blood culture division. In our setting, all patients with suspected bloodstream infections often have their blood cultures ordered in order to begin empirical antibiotic medication. Ordering the cultures in the appropriate instances is not financially restricted because the hospital offers free services.

2.1. AST method

The blood culture isolates of major five gram-negative pathogens were enrolled into the study—three Enterobacterales (ENB) species *Escherichia coli, Klebsiella pneumoniae*, and *Enterobacter cloacae* complex; and two non-fermenters (NFGNB) i.e., *Acinetobacter baumannii* complex, and Pseudomonas aeruginosa. The AST was carried out in by VITEK2 system, using two AST panels—N405 for Enterobacterales, N406 for non-fermenters.[9] The AST method performed was in accordance with the guidelines provided by the manufacturer and the Clinical and Laboratory Standards Institute (CLSI) M100 and M07.[10,11] The laboratory was engaging in an external quality assessment services (EQAS) program and adhering to the weekly AST quality control (QC) methodology, as per CLSI M07.[11,12]

2.2. Data collection

The AST data [minimum inhibitory concentration (MIC) value and interpretation] were collected using 'Clinical Microbiology Reporting software', developed by JIPMER in collaboration with Ibhar Pvt. Ltd., which was routinely used by our institute for reporting of blood culture. The AST report was validated as per CLSI M39.[2] and other standard guideline.[1,11] All erroneous AST data were excluded from analysis and suspicious AST results were reconfirmed before inclusion into antibiogram.

2.3. Routine antibiogram

First, the routine antibiogram was prepared for major five gram-negative pathogens—*E. coli*, *K. pneumoniae*, and E. cloacae complex, *A. baumannii* complex, and *P. aeruginosa.* The 'first-isolate data' were collected; defined as the first-isolate of a given species per patient per analysis period (e.g., one year in our study). The antimicrobial agents included in the routine antibiogram were—cephems (ceftriaxone [for ENB only], ceftazidime [for NFGNB only] and cefepime), β-lactam combination agents (amoxicillin-clavulanate [for *E. coli* and *K. pneumoniae* only], cefoperazone-sulbactam and piperacillin-tazobactam), carbapenems (meropenem, imipenem and ertapenem [for ENB only]), quinolones (ciprofloxacin, levofloxacin [for

NFGNB only],) and aminoglycosides (gentamicin [for all except *P. aeruginosa*] and amikacin), and cotrimoxazole (for all except *P. aeruginosa*). The isolates of an organism species for which results were not available for any of the antimicrobial agents (for several reasons such as intrinsic resistant, no breakpoint available, VITEK2 got terminated etc.) were excluded from analysis. The routine antibiogram was expressed in terms of susceptibility (S%) percentage. Both susceptible (S) and susceptible dose dependent (SDD) results of the first-isolate were taken for numerator, whereas any all the first-isolates tested and valid AST results are available for particular antibiotic were used as denominator data for calculating S%. The antibiogram developed was validated as per CLSI M39 recommendations and any suspicious or outlier S% were reviewed before their inclusion into antibiogram.

2.4. Combination agent antibiogram

The various combination regimens chosen for combination agent antibiogram comprised of one β-lactam agent (cephems or BLBLI or carbapenem), quinolones or aminoglycosides. S% to the combination agents was calculated as % of isolates susceptible to either of the antimicrobial agents. For example, S% to meropenem/amikacin combination included the isolates that were susceptible to meropenem and resistant to amikacin, susceptible to amikacin and resistant to meropenem and susceptible to both meropenem and amikacin. The combination agent antibiogram in the current study was only prepared from common pathogenic GNBs in our settings and also which are common causes nation-wide. These are often MDR in our settings with very less number of single agents having >80% susceptibility and might require combination therapy for empirical choices. Other GNBs are not included in the study as they are encountered rarely as a pathogen in our setting. GPCs were not included in the present study, as almost all of them have good empirical coverage with one single agent and combination empirical therapy is seldom needed. Further stratification of combination agent antibiogram into patientcare locations (e.g., ICUs or IPDs or OPDs), treating specialities (e.g., medicine, surgery etc) and HAIs/CAIs and age wise stratifications etc. were not performed as the minimum number of isolates required to prepare antibiogram at these stratified levels were not met.

2.5. Statistical analysis

Chi-square test was used to analyse the differences in S% between the single antimicrobial agents and to combination regimens, and a p value of < 0.05 was considered statistically significant as recommended in the CLSI M39 and other standard antibiogram references[1,2]. All analyses were conducted using IBM SPSS version 23.

3. Result

For the year 2023, a total 2126 isolates were subjected to AST. After applying the first-isolate per organism species filter, there were 1670 isolates selected which belonged to the five major gram-negative pathogens—i.e. *E. coli* (644), *K. pneumoniae* (389), E. cloacae (85), *P. aeruginosa* (185), and *A. baumannii* (367). Tables 1 to 6 depict the combination agent antibiogram of common antimicrobial classes viz β-lactam drugs, aminoglycosides, fluoroquinolones and folate antagonist for *E. coli* (Table 1), *K. pneumoniae* (Table 2), E. cloacae (Table 3), *P. aeruginosa* (Table 4), *A. baumannii* (Table 5), and all major gram-negative bacilli (Table 6).

Overall, for all the major gram-negative pathogens, the S% of all the combination regimens were found to be higher than the S% of either of the individual agents, however this increase in S% was not statistically significant for most of the instances. The combination regimens where the increase in S% is found to be statistically significant compared to the S% of the individual agents, are highlighted in blue in Tables 1, 2, 3, 4, 5 and 6.

For *E. coli* (Table 1), the S% of gentamicin combination regimens with amoxicillin-clavulanate, cefoperazone-sulbactam, piperacillin-tazobactam, meropenem, imipenem and ertapenem were found to be statistically significant ($p = <0.05$) compared to the S% of either gentamicin alone or the respective individual agents. Similarly, the amikacin combination regimens with cefoperazone-sulbactam, piperacillin-tazobactam, meropenem, imipenem and ertapenem for *E. coli* were found to be statistically significant ($p = <0.05$) compared to the S% of either amikacin alone or the respective individual agents. Likewise, the S% of cotrimoxazole combination regimens with ceftriaxone, cefepime and amoxicillin-clavulanate for *E. coli* were found to be statistically significant ($p = <0.05$) compared to the S% of either cotrimoxazole alone or the respective individual agents.

For *K. pneumoniae* (Table 2), the S% of gentamicin combination regimens with cefoperazone-sulbactam, meropenem, imipenem and ertapenem were found to be statistically significant ($p = <0.05$) compared to the S% of either gentamicin alone or the respective individual agents. Similarly, the cotrimoxazole combination regimens with piperacillin-tazobactam for *K. pneumoniae* was found to be statistically significant ($p = <0.05$) compared to the S% of either cotrimoxazole or piperacillin-tazobactam alone.

For *Enterobacter cloacae* complex (Table 3), the S% of gentamicin combination regimens with imipenem and ertapenem were found to be statistically significant ($p = <0.05$) compared to the S% of either gentamicin alone or the respective individual agents. The S% increase of rest of all combination regimens were found to be statistically insignificant.

For *P. aeruginosa* (Table 4), and *A. baumannii* complex (Table 5), none of the combination regimens were found to have statistically significant S% (p= >0.05) compared to the S% of the respective individual agents.

For major gram-negative pathogens (Table 6), several combination regimens were found to have statistically significant S% (p ≤0.05) compared to the S% of the respective individual agents. The list has been highlighted blue in the Table-6, which include—ceftriaxone combination regimen with ciprofloxacin; cefepime combination regimens with gentamicin or cotrimoxazole; amoxicillin-clavulanate combination regimens with ciprofloxacin or cotrimoxazole; cefoperazone-sulbactam combination regimens with gentamicin, amikacin, or cotrimoxazole; piperacillin-tazobactam combination regimens with gentamicin or cotrimoxazole; meropenem combination regimens with gentamicin; imipenem combination regimens with gentamicin, amikacin, or cotrimoxazole; ertapenem combination regimens with gentamicin, ciprofloxacin or cotrimoxazole; gentamicin combination regimens with ciprofloxacin or cotrimoxazole; amikacin combination regimens with cotrimoxazole; ciprofloxacin combination regimens with gentamicin or cotrimoxazole and cotrimoxazole combination regimens with gentamicin, amikacin, or ciprofloxacin.

4. Discussion

In suspected gram-negative bacterial infections, using a combination of two distinct antimicrobial drugs as empirical therapy may be necessary, especially when the prevalence of multidrug resistant bacteria is high. In such cases, clinicians select the agents for double gram-negative coverage based on traditional antibiograms. These conventional antibiograms provide S% data of single antimicrobial agents, however, do not accurately depict the combined probability of S% when two antimicrobial drugs are used together. combination agent antibiogram s serve this purpose of assisting the clinical team in choosing the right combinations of antimicrobials. Our study represents first-of-its kind study from India, depicting the combination agent antibiogram of common antimicrobial classes viz β-lactam drugs, aminoglycosides, fluoroquinolones and folate antagonist for major gram-negative bacterial pathogens.[1,13]

4.1. Combination agent antibiogram for E. coli

For *E. coli*, several combination regimens using one β-lactam agent plus either aminoglycoside or cotrimoxazole were found to have statistically significant increase in S% compared to the S% of individual agents (Table 1). The multidrug resistance (MDR) rates in *E. coli* isolates included in our study was found to be 73.7% which is quite high. However, the extensive drug resistance (XDR) rate was comparatively low, i.e. only 7.6%. This might be the reason why we have observed significant improvement in S% for combination regimens as compared to individual drugs. This provides several options of combination regimen for *E. coli* as empirical choice. While cotrimoxazole combination regimens were found to be useful when combined with lower line β-lactams such as ceftriaxone, cefepime and amoxicillin-clavulanate; the aminoglycoside combination regimens were found beneficial when combined with higher line BLBLI such as cefoperazone-sulbactam and piperacillin-tazobactam, or carbapenems such as meropenem, imipenem, ertapenem.[7,14]

E. coli is usually the most common gram-negative pathogen isolated from clinical specimens, as in our study. Therefore, when intended to choose a gram-negative empirical regimen, the prescribers often incline towards choosing an agent that gives a broad coverage to *E. coli*. Moreso, empirical regimen for *E. coli* is used by the clinicians in two clinical situations—(1) when the identification of the organism is known but AST report is awaited; (2) in clinical situations where *E. coli* is primarily suspected as the underling pathogen (e.g. community- or healthcare associated urinary tract infection, urosepsis, etc.). Having a knowledge on the estimated S% of various combination regimens will definitely guide the clinicians to choose the correct combination of antimicrobial agents in the regimen.[3,13] Based on the results of our study, it can be proposed that, the clinicians in our facility, who intend to select a combination regimen with one of the lower line β-lactams such as ceftriaxone or cefepime, or amoxicillin-clavulanate as the first agent, should prefer to add cotrimoxazole as the second agent. But for hemodynamically unstable or severely-ill patients, if clinicians decide to choose a higher line β-lactam agent such as cefoperazone-sulbactam and piperacillin-tazobactam, or carbapenems, then the second agent added should be an aminoglycoside in order to give a maximum increase in expected S% of the combined regimen.[15,16]

4.2. Combination agent antibiogram for K. pneumoniae

For *K. pneumoniae*, in contrast to *E. coli,* only a limited combination regimens were found to be statistically significant increase in S% compared to the S% individual agents. If cefoperazone-sulbactam or any of the carbapenems as the β-lactam is used, then the second agent selected in the combination regimen should be gentamicin. Whereas combination regimens using piperacillin-tazobactam should use cotrimoxazole as the second agent. In our facility, the XDR rates in the *K. pneumoniae* isolates included in our study was found to be 39.3%, which is exuberantly high compared to *E. coli*. This might be the reason why the combination regimens were not found to be significantly effective with respect to the monotherapy, as compared to that in *E. coli*.[17,18]

Table 1: Combination agent antibiogram of common antimicrobial classes viz β-lactam drugs, aminoglycosides, fluoroquinolones and folate antagonist for *Escherichia coli* (N=644)

Antimicrobial agents	Monotherapy S%(n)	Combination therapy S%(n) with Gentamicin	Amikacin	Ciprofloxacin	Cotrimoxazole
Ceftriaxone	18.0 % (116)	60.4% (389)	81.2%(523)	18.9%(122)	42.7%(275)
Cefepime	38.8 % (250)	65.4%(421)	82.0%(528)	39.3%(253)	55.0%(354)
Amoxicillin-clavulanate	43.2%(278)	68.2%(439)	82.3%(530)	43.5%(280)	57.1%(368)
Cefoperazone-sulbactam	75.6%(487)	86.8%(559)	89.0%(573)	75.6%(487)	78.4%(505)
Piperacillin-tazobactam	68.3%(440)	83.4%(537)	86.5%(557)	68.3%(440)	74.5%(480)
Meropenem	79.0%(509)	89.0%(573)	90.1%(580)	79.3%(511)	80.4%(518)
Imipenem	80.3%(517)	89.8%(578)	90.2%(581)	80.3%(517)	81.7%(526)
Ertapenem	78.9%(508)	88.7%(571)	89.9%(579)	79.0%(509)	80.6%(519)
Gentamicin	59.2%(381)	NA	NA	59.2 %(381)	68.0 %(438)
Amikacin	81.1%(522)	NA	NA	81.1%(522)	84.6 %(545)
Ciprofloxacin	3.9%(25)	59.2%(381)	81.1%(522)	NA	36.0 %(232)
Cotrimoxazole	35.2%(227)	68.0%(438)	84.6%(545)	36.0%(232)	NA

Note: Cells highlighted blue are indicative of significant increase in S% of the combination regimen compared to the S% of the individual single agent.

Table 2: Combination agent antibiogram of common antimicrobial classes viz β-lactam drugs, aminoglycosides, fluoroquinolones and folate antagonist for *Klebsiella pneumoniae* (N= 389)

Antimicrobial agents	Monotherapy S%(n)	Combination therapy S%(n) with Gentamicin	Amikacin	Ciprofloxacin	Cotrimoxazole
Ceftriaxone	27.8%(108)	53.2%(207)	53.7%(209)	30.8%(120)	44.5%(173)
Cefepime	36.5%(142)	55.8 %(217)	53.7%(209)	37.0 %(144)	48.6 %(189)
Amoxicillin-clavulanate	33.4%(130)	53.7%(209)	54.2%(211)	36.0%(140)	49.4%(192)
Cefoperazone-sulbactam	49.9 %(194)	61.7 %(240)	54.5%(212)	50.4 %(196)	57.6 %(224)
Piperacillin-tazobactam	45.8%(178)	59.1%(230)	54.0%(210)	46.5%(181)	54.8%(213)
Meropenem	51.2 %(199)	62.5 %(243)	55.8%(217)	51.7 %(201)	58.1 %(226)
Imipenem	51.4%(200)	63.0%(245)	55.8%(217)	51.9%(202)	58.6%(228)
Ertapenem	49.9 %(194)	61.7 %(240)	54.5%(212)	50.9 %(198)	57.8 %(225)
Gentamicin	53.0%(206)	NA	NA	54.8 %(213)	59.6 %(232)
Amikacin	53.7 %(209)	NA	NA	54.0 %(210)	60.2 %(234)
Ciprofloxacin	26.7%(104)	54.8 %(213)	54.0%(210)	NA	44.7 %(174)
Cotrimoxazole	43.4 %(169)	59.6 %(232)	60.2(234)	44.7 %(174)	NA

Note: Cells highlighted blue are indicative of significant increase in S% of the combination regimen compared to the S% of the individual single agent.

4.3. Combination agent antibiogram for Enterobacter cloacae complex

For *Enterobacter cloacae* complex (Table 3), the only combination regimen that was found to have statistically significant increase in S% was 'imipenem or ertapenem plus gentamicin'. MDR rate in E. cloacae was found to be low (42.9%) in our center as compared to *E. coli* (73.7%) and *K. pneumoniae* (71.6%), therefore, coverage with monotherapy is often found to be effective and combination regimen may not be required.[16] As per the statistical table for increase in S% provided in CLSI M39, when the sample size is low, only a higher increase in S% makes it statistically significant, while with a high sample size, even a lower increase in S% makes it statistically significant. As in the present study, the sample size for E. cloacae complex was relatively low (i.e. 85), as compared to *E. coli* (644) and *K. pneumoniae* (389), this might be the reason that lower number of combination regimens with statistically significant increase in S% were observed in E. cloacae complex, as compared to *E. coli* and *K. pneumoniae*.[2,17,18]

4.4. Combination agent antibiogram for P. aeruginosa and A. baumannii complex

Unfortunately, our study did not find a single combination regimen useful for *P. aeruginosa* (Table 4), and *A. baumannii* complex (Table 5), as none of the combination

Table 3: Combination agent antibiogram of common antimicrobial classes viz β-lactam drugs, aminoglycosides, fluoroquinolones and folate antagonist for *Enterobacter cloacae* complex (N= 85)

Antimicrobial agents	Monotherapy S%(n)	Combination therapy S%(n) with			
		Gentamicin	Amikacin	Ciprofloxacin	Cotrimoxazole
Ceftriaxone	49.4% (42)	74.1% (63)	85.9%(73)	60.0% (51)	76.5% (65)
Cefepime	75.3% (64)	83.5% (71)	91.8%(78)	77.6% (66)	83.5% (71)
Cefoperazone-sulbactam	81.2% (69)	91.8% (78)	87.1%(74)	83.5% (71)	88.2% (75)
Piperacillin-tazobactam	72.9% (62)	85.9% (73)	87.1%(74)	76.5% (65)	84.7% (72)
Meropenem	83.5% (71)	91.8% (78)	87.1%(74)	83.5% (71)	88.2% (75)
Imipenem	80.0% (68)	91.8% (78)	87.1%(74)	81.2% (69)	87.1% (74)
Ertapenem	78.8% (67)	90.6% (77)	87.1%(74)	82.4% (70)	87.1% (74)
Gentamicin	74.1% (63)	NA	NA	75.3% (64)	83.5% (71)
Amikacin	85.9% (73)	NA	NA	85.9% (73)	91.8% (78)
Ciprofloxacin	55.3% (47)	75.3% (64)	85.9%(73)	NA	77.6% (66)
Cotrimoxazole	75.3% (64)	83.5% (71)	91.8%(78)		NA

Note: Cells highlighted blue are indicative of significant increase in S% of the combination regimen compared to the S% of the individual single agent.

Table 4: Combination agent antibiogram of common antimicrobial classes viz β-lactam drugs, aminoglycosides and fluoroquinolones for Pseudomonas aeruginosa (N=185)

Antimicrobial agents	Monotherapy S%(n)	Combination therapy S%(n) with		
		Amikacin	Ciprofloxacin	Levofloxacin
Ceftazidime	74.6% (138)	79.5%(147)	77.8% (144)	76.2% (141)
Cefepime	73.5% (136)	77.8%(144)	76.2% (141)	75.7% (140)
Cefoperazone-sulbactam	72.4% (134)	77.8%(144)	76.2% (141)	74.6% (138)
Piperacillin-tazobactam	71.4% (132)	77.3%(143)	75.1% (139)	73.5% (136)
Meropenem	77.3% (143)	80.5%(149)	78.9% (146)	78.4% (145)
Imipenem	77.3% (143)	82.2%(152)	80.0% (148)	78.9% (146)
Amikacin	76.2% (141)	NA	76.8% (142)	76.8% (142)
Ciprofloxacin	68.1% (126)	76.8%(142)	NA	NA
Levofloxacin	63.2% (117)	76.8%(142)	NA	NA

Note: S% increase of none of the combination regimen was found to be statistically significant compared to the S% of the individual single agent, therefore, no cells have been highlighted.

Table 5: Combination agent antibiogram of common antimicrobial classes viz β-lactam drugs, aminoglycosides, fluoroquinolones and folate antagonist for *Acinetobacter baumannii* complex (N=367)

Antimicrobial agents	Monotherapy S%(n)	Combination therapy S%(n) with				
		Gentamicin	Amikacin	Ciprofloxacin	Levofloxacin	Cotrimoxazole
Ceftazidime	16.3% (60)	19.9% (73)	20.2%(74)	19.6% (72)	19.6% (72)	28.1% (103)
Cefepime	16.1% (59)	19.3% (71)	19.1%(70)	18.5% (68)	18.5% (68)	27.2% (100)
Cefoperazone-sulbactam	23.7% (87)	25.9% (95)	25.9%(95)	25.1% (92)	24.8% (91)	31.9% (117)
Piperacillin-tazobactam	16.9% (62)	20.2% (74)	19.9%(73)	19.3% (71)	19.3% (71)	27.8% (102)
Meropenem	17.4% (64)	20.4% (75)	20.2%(74)	19.9% (73)	19.9% (73)	28.3% (104)
Imipenem	16.9% (62)	20.2% (74)	19.9%(73)	19.6% (72)	19.6% (72)	28.1% (103)
Gentamicin	18.8% (69)	NA	NA	20.7% (76)	20.7% (76)	18.8% (69)
Amikacin	18.5% (68)	NA	NA	21.5% (79)	21.3% (78)	29.4% (108)
Ciprofloxacin	17.2% (63)	20.7% (76)	21.5%(79)	NA	NA	28.3% (104)
Levofloxacin	17.2% (63)	20.7% (76)	21.3%(78)	NA	NA	28.3% (104)
Cotrimoxazole	26.7% (98)	18.8% (69)	29.4%(108)	28.3% (104)	28.3% (104)	NA

Note: S% increase of none of the combination regimen was found to be statistically significant compared to the S% of the individual single agent, therefore, no cells have been highlighted.

Table 6: Combination agent antibiogram of common antimicrobial classes viz β-lactam drugs, aminoglycosides, fluoroquinolones and folate antagonist for major five gram-negative bacilli; i.e. *E. coli*, *K. pneumoniae*, E. cloacae, *P. aeruginosa* and *A. baumannii* (N=1670)

Antimicrobial agents	Monotherapy	Combination therapy with			
		Gentamicin	Amikacin	Ciprofloxacin	Cotrimoxazole
Ceftriaxone	15.9% (266)	43.6%(728)	60.7%(1014)	28.9% (482)	36.6% (611)
Cefepime	39.0% (651)	54.9%(916)	61.6%(1029)	40.2% (672)	50.9% (850)
Amoxicillin-clavulanate	24.4% (408)	46.7%(780)	61.3%(1023)	39.3% (656)	43.2% (722)
Cefoperazone-sulbactam	58.1% (971)	66.2%(1106)	65.7%(1098)	59.1% (987)	63.2% (1055)
Piperacillin-tazobactam	52.3% (874)	62.6%(1046)	63.3%(1057)	53.7% (896)	59.8% (999)
Meropenem	59.0% (986)	66.6%(1112)	65.5%(1094)	60.0% (1002)	63.8% (1066)
Imipenem	59.3% (990)	66.9%(1118)	65.7%(1097)	60.4% (1008)	64.4% (1076)
Ertapenem	46.0% (769)	57.3% (957)	64.3%(1074)	57.8% (966)	54.9% (916)
Gentamicin	45.0% (751)	NA	NA	51.5% (860)	50.8% (849)
Amikacin	60.7%(1013)	NA	NA	61.4% (102)	66.2% (1106)
Ciprofloxacin	21.9% (365)	51.5% (860)	61.4%(1026)	NA	42.0% (702)
Cotrimoxazole	33.4% (558)	50.8% (849)	66.2%(1106)	42.0% (702)	NA

Note: Cells highlighted blue are indicative of significant increase in S% of the combination regimen compared to the S% of the individual single agent. Ceftazidime, and levofloxacin were not tested for Enterobacterales, hence excluded from analysis

regimens were found to have statistically significant S% ($p \leq 0.05$) compared to the S% of the respective individual agents.[4] This is because of the occurrence of a high rate of drug resistance in *P. aeruginosa* (26% XDR), and *A. baumannii* complex (55.9% XDR), in our facility. XDR isolates are resistant to most of the antimicrobial classes and therefore will not be covered even by combination regimens. Therefore, use of combination regimen does not give any added advantage over monotherapy for suspected *P. aeruginosa* and *A. baumannii* complex infection, and therefore should not be used empirically.[2,8,14]

4.5. Combination agent antibiogram at GNB-level

When prepared the combination agent antibiogram at GNB-level, based on the joined data of five major gram-negative pathogens, i.e. *E. coli*, *K. pneumoniae*, *E. cloacae, P. aeruginosa* and *A. baumannii*. It was observed that several combination regimens were found to have statistically significant S% ($p = <0.05$) compared to the S% of the respective individual agents. The higher statistical significance in GNB analysis is mainly attributed to the higher number of isolates (1640) subjected to analysis. When the isolate number is very high (e.g. >1000), even a smaller increase in S% is found to be statistically significant.[2]. However, for a smaller number of isolates, only a higher increase in S % makes it statistically significant.[2] Even if antimicrobial empirical combinations which had statistically significant S% were not found for *P. aeruginosa* and *A. baumannii* complex, still the combination agents demonstrated relatively better S% than individual agents and hence would still help as combination empirical choices as compared to individual monotherapy.

The gentamicin combination therapy was found to be statistically significant for majority of β-lactams, except for ceftriaxone and amoxicillin clavulanate. Ceftriaxone and amoxicillin clavulanate are in general not a good empirical agent for suspected gram-negative infection as several major gram-negative pathogens like Ps and Ac are IR to these agents. Gentamicin monotherapy is as such not recommended for bloodstream infections. However, in the current study we found that it gives an added advantage in S% when combined with combination therapy fourth generation cephems, higher BLBI and carbapenems; hence gentamicin combination therapy is a good choice for empirical therapy.[14]

Cotrimoxazole combination therapy with β-lactams is rarely a preferred combination regimen as empirical therapy for suspected gram-negative sepsis. However, in the current study, we have found a significant improvement in S% when cotrimoxazole is combined with most of the β-lactams agents. Therefore, we urge to keep cotrimoxazole combination therapy with β-lactams as empirical option for suspected gram-negative infections. Our study found ciprofloxacin combination therapy with β-lactams is a less useful regimen as in most of the instances, the increase in S% in not significant.[1,8,15]

Combination agent antibiogram can be particularly useful in settings where — (i) high-risk patients like immunocompromised, transplant recipients, etc. are common; (ii) S% of individual agents are less than optimal to be a good empirical choice as monotherapy; (iii) S% of different organisms against various antimicrobial agents grossly vary from each other and therefore a combination therapy is preferred to provide better coverage.[16] However, the S% obtained in combination agent antibiogram is not derived from any in vitro synergy testing, and the

potential synergistic or antagonistic interactions between the individual agents are unknown. More so, higher S% to combination agents does not indicate that the two agents given together is necessarily more effective than either of the agent given as monotherapy.[4] It only indicates that the chance of the suspected organism being susceptible is higher when given in combination than as monotherapy, and therefore there is a higher likelihood of therapeutic success. Such kind of combination regimen is only meant for empirical therapy and must be de-escalated (monotherapy) to the most possible narrow-spectrum agent once the culture and AST report are available.[7,17–19]

5. Conclusion

In hemodynamically unstable or critically sick patients, prescribers have a tendency to empirically start on combination antimicrobial regimens instead of monotherapy. However, most of these choices made are based on their own experience, but not evidence-based. Combination agent antibiogram for empirical choice is an excellent tool to provide evidence-based recommendations on selecting correct combination of antimicrobial agents as empirical choices. In the current study, many such combination regimens have been found to demonstrate statistically significant improvement in S% for *E. coli* or *K. pneumoniae* or at GNB level, but not for *P. aeruginosa*, *A. baumannii* or E. cloacae. Every healthcare facility should prepare combination agent antibiogram for the most commonly used antimicrobial combination regimens after discussion with the prescribers.

6. Ethical Approval

This study was conducted after taking approval from Ethical approval committee of Institute with ref. no. JIP/IEC/2021/256

7. Conflict of Interest

None.

8. Source of Funding

None.

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