Tackling Multidrug Resistance in Neonatal Sepsis: A South Asia Perspective

<table>
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<tr>
<th>Journal:</th>
<th>BMJ</th>
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<tbody>
<tr>
<td>Manuscript ID</td>
<td>BMJ.2018.045301</td>
</tr>
<tr>
<td>Article Type:</td>
<td>Analysis</td>
</tr>
<tr>
<td>BMJ Journal:</td>
<td>BMJ</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>31-May-2018</td>
</tr>
</tbody>
</table>
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<p>| Keywords:    | Neonatal Sepsis Multidrug Resistance South Asia Early-onset Late-onset |</p>
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<th>Title</th>
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Title: Tackling Multidrug Resistance in Neonatal Sepsis: A South Asia Perspective

Standfirst: Sepsis caused by multidrug resistant pathogens has emerged as a major threat to neonatal survival and well-being in South Asia and carries serious global implications. Suman Chaurasia and colleagues present a situational analysis, and discuss the issues and the way forward.

Abstract
Sepsis continues to be the third most common cause of deaths among the neonates across the world. Most of these deaths occur in developing countries settings, with South Asia region alone accounting for around 40% of global burden. Recent regional trends indicate soaring antimicrobial resistance among the causative organisms- a major threat to neonatal survival and well-being in South Asia with potential global implications. We conducted a systematic review to perform a situational analysis and discuss the way forward.

The systematic review highlighted similar rates of culture-positive sepsis still persisting over the decades, compounded by the recent alarming presence of multidrug resistant organisms (MDROs) in the region. Pertinent questions that emerged were probed with the aid of Delhi Neonatal Infection Study (DeNIS) collaboration’s database and led to a three-pronged critical approach, overlapping with existing (inter)national recommendations. Setting up a diagnostic-focussed antibiotic stewardship mass movement will aid in slowing the evolution of MDROs; elucidating transmission pathways will prevent exposure/infection due to the latter; and establishing a regional dedicated clinical drug trial network in collaboration with multiple stakeholders will potentially ensure alternative treatment options against MDRO caused sepsis.
Key Messages

- The incidence of sepsis in neonates in South Asia region has remained unchanged but the proportion of sepsis caused by multidrug resistant organisms (MDROs) has risen sharply potentially leading to higher mortality burden.

- This calls for concerted efforts to slow the evolution of MDROs by strengthening antibiotic stewardship in health facilities alongside devising effective point-of-care diagnostics; preventing the MDRO-infections by first understanding and then interrupting their transmission pathways; and finding effective drugs and adjuvant care modalities by building dedicated regional clinical trials network.

Word count: 2278/2200
Introduction

Systemic infection or sepsis in the neonatal period – the first 28 days of life – is the third most common cause of deaths among neonates around the world.¹ Neonatal sepsis includes bloodstream infection, meningitis, and pneumonia. It is usually categorized into ‘early-onset’ sepsis (EOS; onset within 72h of birth) and ‘late-onset’ sepsis (LOS; onset beyond 72h). The former is considered to be caused by pathogens vertically transmitted from mother while the latter is due to pathogens acquired horizontally from environment and/or caregivers. Sepsis is also classified as culture-positive or culture-negative depending upon isolation of pathogen(s) from blood or other sterile fluids. Of the global burden of sepsis-related neonatal deaths, nearly 40% occur in resource-limited countries of South Asia (SA: Afghanistan, Bangladesh, Bhutan, India, Maldives, Nepal, Pakistan, and Sri Lanka).¹

Neonates as immunocompromised hosts are more prone to infections. Thus, antibiotics are among the commonest drugs to be used in NICUs.² The resultant antibiotic selection pressure leads to development of antimicrobial resistance (AMR).³ Recent reports indicate an alarming level of multidrug resistance (MDR) among common pathogens of neonatal sepsis in the region.⁴ Every year, around 56500 neonatal deaths are attributable to sepsis caused by pathogens resistant to first-line drugs in India alone.⁶ Resistance elements like CTX-M-15 and NDM-1 genes may have disseminated globally after originating from SA.⁷⁸ In this article, we discuss AMR patterns in neonatal sepsis in SA, contextual issues and perspectives.

Neonatal sepsis in South Asia
We conducted a systematic review of literature published in PubMed between January 2000 and April 2018 using search terms (newborn OR neonate) AND (sepsis OR infection OR antibiotic OR antimicrobial) (Supplementary Panel 1). After filtering for countries in SA, the search strategy yielded a total of 2536 studies. Among the 105 studies finally identified (India=68; Pakistan=15; Bangladesh=7; and Nepal=14), we found more than one unique cohorts/epochs datasets within the studies and treated them separately, making a total of 114 reports. We also performed secondary analysis from our Delhi Neonatal Infection Study (DeNIS) database, as and when necessary.9

**Incidence and onset of neonatal sepsis**

The incidence of culture-positive sepsis was 17.8 per 1000 live births (95% CI 14.3-21.2, n=17 reports; Table 1). In community settings, it was lower - varying from 2.910 to 6.711 per 1000 live-births. The incidence of culture-positive sepsis in SA hardly changed from that in 2005 (15 per 1000 live-births),5 and remained 3-4 fold higher than that (1-5 per 1000 live-births) reported in high income countries (HICs).5 EOS constituted around two-thirds (62.3 %; 61.4-63.0) of total sepsis.

**Pathogen profile and antimicrobial resistance pattern**

Among all isolates (n=24,273) from SA region, Gram-negative organisms (63%) were the most common pathogens, with *Klebsiella* spp. (22.3%), *Escherichia coli* (13.9%) and *Acinetobacter* spp. (7.9%) being the top three. The common Gram-positive organisms were *Staphylococcus aureus* (19.9% of total isolates) and coagulase negative staphylococci (8.5%). There was a striking similarity between the pathogen profile of EOS and LOS.

Overall, the common organisms were *Klebsiella* spp., *Staphylococcus aureus, Escherichia coli,* and *Acinetobacter* spp., and they uniformly exhibited high degree of resistance to WHO
recommended first-line drugs namely, ampicillin (weighted median 69% to 88.2%) and gentamicin (54.5% to 75.3%), as well as third generation cephalosporins like cefotaxime (51.2% to 72.5%) (Table 2). However, most Gram-negative isolates were susceptible to WHO-classified ‘Watch Group’ antibiotics like meropenem (carbapenems), colistin, and tigecycline (Supplementary Table 1). About a half (weighted proportion 46.5% [41.9-51.1]) of Staphylococcus aureus isolates were methicillin resistant (MRSA) but most remained susceptible to WHO-classified ‘Watch Group’ as vancomycin and linezolid. Available AMR rates against first-line antibiotics (ampicillin: 81%-82%; gentamicin: 55-74%) and cefotaxime (51%-54%) of Klebsiella spp and E. coli indicate the pattern seemed to have remained almost unchanged over the decade. A recent systematic review from India reported similar AMR rates among Gram-negative organisms.

Multidrug resistance amongst pathogens

The limited number of publications and heterogeneity in definitions of MDR made it challenging to draw inferences (Supplementary Table 2). The most common used definition for MDR was resistance to 2-3 or more antimicrobial agents/classes, usually involving first-line and sometimes second-line drugs. An experts panel defined extreme-drug-resistance (XDR) as resistance to all categories but one or two antibiotics tested. Recent reports used presence of extended spectrum beta-lactamase (ESBL- a key enzyme inducing MDR) in the pathogens as a surrogate for MDR. MDR rates in Klebsiella spp., Escherichia coli and Acinetobacter spp. (3-4 reports for each) were 70.7% (95% CI 66.1-75.3), 54.0% (48.1-59.9), and 78.7% (73.9-83.4), respectively (Table 2). Notably, all these studies were published in the last five years. The ESBL rates among Klebsiella spp., Escherichia coli and Acinetobacter spp. were 53.8% (95% CI 50.9- 56.6, n= 25), 42.4% (38.6-46.2, n= 18) and 65.6% (55.9-75.3, n=3) respectively (Table 1). Of the studies
reporting XDR (n=3-4 for each), rates were 5.2% (95% CI 2.5-7.8) in *Klebsiella* spp. 2.9% (0.7-5.2) in *E. coli* and 17.5% (13.1-21.9) in *Acinetobacter* spp.

Comparing EOS with LOS (n=3), MDR rates in EOS and LOS were similar amongst *Klebsiella* spp., *E. coli* and *Acinetobacter* spp. (Table 2). However, in the study that provided most isolates and more robust data, the MDR rate was higher in EOS by nearly 10 percentage points (in top two pathogens- *Klebsiella* spp. and *E. coli*).

Among Gram-positive organisms, about half of *S. aureus* isolates labelled methicillin resistance (see above) are generally equated to MDR. A recent systematic review also noted that 50% of *S. aureus* isolates were methicillin-resistant.

*Case fatality rates*

The median case fatality rate (proportion of deaths attributable to sepsis among cases of sepsis; CFR) for culture-positive sepsis was 34.4% (IQR 33.1 to 35.6, n= 19; Table 1). It was higher for EOS than LOS; 45.4% (42.2 to 48.6, n= 9) vs. 26.4 % (23.6 to 29.2, n= 6).

The median CFR among the three commonest Gram-negative MDR pathogens was slightly higher than non-MDR counterparts: 59.8% (95% CI 54.5 to 65.2) vs. 55.8% (49.1 to 62.6). It was nearly double for ESBL-producing pathogens than for non-ESBL-producers (27.3% vs. 15.6%).

*Key findings and inferences regarding sepsis in South Asia*

The incidence and case fatality of culture-positive sepsis continues to be high in SA. The bacterial pathogen profile is similar across the region with a predominance of MDR organisms (MDROs). EOS comprises of two thirds of all cases, with its pathogen profile resembling that of
classical LOS. Given its larger share and higher case fatality rates than LOS, EOS carries a greater epidemiological significance in SA.

High degree of MDR with evidence suggesting its recent emergence in the region is quite alarming. In contrast to LOS, MDR in EOS is greater and associated with high CFR. The remarkable degree of resistance exhibited by the pathogens to most antibiotics leaves clinicians grappling for treatment options.

**Knowledge gaps, insights/analyses from DeNIS and perspectives**

With this recap, several pertinent gaps in knowledge seek our attention:

1. What is the reason behind recent emergence of MDRO and how can we mitigate the evolution of MDRO?
2. What are the source and routes of transmission of MDROs in EOS so as to guide effective preventive strategies?
3. Lastly, do existing regimens still hold value to treat infection due to MDROs? And, how best new or alternative treatment options can be devised in LMICs?

We probed the DeNIS database to better understand the issues and navigate the way forward. For the purpose of this article, we limited the scope of discussion to these burning questions.

I. *Mitigating evolution of MDR organisms by enhanced antibiotic stewardship:* The evolution of MDRO in SA is likely to be multi-factorial. Here widespread antibiotic residues generated from agriculture and pharmaceutical industries have generally contaminated the environment, including that of health sector (Panel 1). Additionally, prescribing antibiotics for sick neonates in facilities carries inherent complexities. Since initial clinical features of sepsis are non-specific and underlying CFR is high, the treating physicians invariably end up initiating
empiric broad-spectrum antibiotics in neonates whenever confronted with sickness.\(^5\)

Consequently, susceptible organisms in neonatal surroundings face multi-dimensional selection pressure to evolve as MDROs.\(^{17}\)

Since environmental factors are outside the purview of the current article, we examined the antibiotic prescription rates in DeNIS study. Of all neonates suspected to have sepsis and initiated on antibiotics, a quarter of them was finally proved not to have sepsis at all and another quarter had culture-negative sepsis (Panel 2, Item 1a). So, antibiotics could have been potentially stopped early (in ‘no sepsis’ cases) or given for a shorter duration (in culture-negative cases) in at least half of the neonates. A point-of-care reliable biomarker can effectively guide antibiotic prescription and in turn, address high selection pressure. Such use of diagnostic modalities is now integral to structure of antibiotic stewardship program (ASP) - a multi-intervention tool to achieve rationalized antibiotic usage.\(^{18}\)

In this context, some upcoming biomarkers like procalcitonin can guide both initiation and early discontinuation of antibiotics.\(^{19,20}\) However, robust evidence for diagnostic accuracy and cost-effectiveness of biomarkers for neonatal sepsis are generally lacking in LMICs. Besides curtailing antibiotic consumption effectively,\(^{21,22}\) ASP has now demonstrated reduction in emergence of MDR Gram-negative organisms in hospitals, by as much as 51% in HICs, with synergistic benefits of simultaneous infection prevention and control (IPC) measures especially hand hygiene.\(^{23}\) However, challenges in LMICs for implementing an effective ASP are manifold besides lack of such evidence base.\(^{24}\) Among the major barriers are, the fixed beliefs and attitudes of the care-providers- requiring integration of behavioural change approaches in the implementation since the outset.\(^{18}\) More importantly, objective
uniform benchmarking outcome for ASP-based audits (such as *C. difficile* reduction rates used in HICs) is lacking. Therefore, there is an urgent need to address the issues around diagnosis and implementation of ASP to slow MDROs fostering in SA.

II. *Determining transmission dynamics (particularly for EOS) to prevent MDROs’ exposure/infection.* In HICs, a clear evidence that Group B streptococci, the predominant pathogen in EOS, get vertically transmitted from colonized mothers to their neonates culminated into instituting intrapartum antibiotics prophylaxis to high risk mothers. Consequently, this strategy led to a major reduction in EOS in HICs. Despite the dominance of EOS in SA, the transmission dynamics of pathogens- increasingly MDROs- remain unresolved.

The current review underscored EOS pathogen profile bore resemblance to classical healthcare (LOS) associated pathogens suggesting horizontal transmission. We found no variation in the pathogen profiles in 12h and 24h time epochs after birth until discharge in DeNIS collaboration study. On the other hand, a quarter of culture-positive sepsis was diagnosed by 24h of birth, with nearly 10% occurring before 12h (Panel 2, Item 1b), potentially too short time for manifesting EOS by horizontal transmission (through colonization and then invasion). Given that EOS manifested so early, and was associated with high degree of MDR as well as case fatality, primary prevention seems to be the logical intervention of choice. But, to enable preventive strategy, the following crucial questions call for urgent answers: do the MDROs colonize the maternal genital tract and consequently are vertically transmitted to their babies during delivery? Or is there simply an ‘ultra-early’ horizontal transmission occurring in birthing rooms and NICUs? Or, a mix of both? Studies
addressing this issue in LMIC are scanty - mostly involving phenotypic correlation of colonizing and invasive isolates.\textsuperscript{26,27} Hence, high quality concordance studies - integrating metagenomic with conventional approaches\textsuperscript{28} are essential to elucidate the transmission pathways and devise optimum preventive strategies.

III. Improving clinical management of MDR sepsis: Using DeNIS database, we compared CFRs of culture-positive sepsis amongst neonates whether or not clinicians upgraded antibiotics from first-line (defined according to prevailing practices across participating units: amikacin or ciprofloxacin or ceftriaxone or piperacillin-tazobactam) after at least 48h of therapy to second-line (vancomycin or meropenem or cefoperazone+ sulbactam). We classified these neonates into three categories: (i) when first-line antibiotics alone covered the isolated pathogen (‘concordant’ group); (ii) when first- did not but second-line covered the pathogen (‘partially discordant’) and (iii) when neither first- nor second-line antibiotics covered the pathogen (‘fully discordant’). We observed a serially increasing order of CFR from first to third categories (23.9%, 31.6% and 57.3% respectively; Panel 2, Item 1c), even in EOS. The fact that worst outcome is associated with fully discordant group, clearly underscores right choices of antibiotics are vital.

In this context, novel antibiotics, active against carbapenem-resistant organisms, such as plazomicin and ceftazidime/avibactam are on the horizon; but, currently high costs (of around $100/day) preclude their use in LMICs.\textsuperscript{29} Recycling of older drugs such as colistin or polymixin B or tigecycline have been explored and proved life-saving in our settings. However, barring reports of off-label usage, hardly any pharmacokinetic or pharmacodynamic data of these drugs are available to guide dosing decisions in neonates.\textsuperscript{30}

Global Antibiotic Research & Development Partnership (GARDP) and Pediatric trial
network are few organizations working in this priority area.\textsuperscript{31,32} Largely, there is an overarching need to develop a committed paediatric/neonatal clinical trials platform in the region evaluating range of interventions including novel, older off-patent or combination antibiotics, and supportive care, besides IPC measures.\textsuperscript{33} This platform, along with the proposed three-dimensional strategy, would indeed act as complementary to comprehensive AMR surveillance, and other supplementary modalities suggested in various national and global action plans.\textsuperscript{34-36}

Conclusions

Our article highlights the virtually unchanged sepsis patterns over the decades and steadily increasing multidrug resistance among neonates in South Asia. We present analyses and insights, and a promising blueprint towards curbing the menace: implementing effective antibiotic stewardship campaign with focus on point-of-care diagnostics to slow MDROs’ evolution; understanding and attacking transmission pathways of early-onset sepsis to prevent it; and establishing a dedicated clinical trials network employing regional data to find best suited treatment alternatives for LMICs. This three-pronged strategy calls for concerted efforts of multiple stakeholders to jointly engage in collaborative research and multiple strategic trials for informing future initiatives and policy-framing.
Contributors and sources The authors work in academic institutions of India (AIIMS, New Delhi) and the UK (SGU London), and non-profit organization (DNDi), and are engaged in collaborative research for prevention and management of sepsis in neonates.

SC, MJS and RA conceived the idea of the paper with inputs from MS, SS and SE. SS conducted the literature search, and extracted the data for systematic review with help from SC. SC wrote the first draft of the manuscript. All the authors contributed to writing/editing various sections, critically reviewing the results, finalizing the draft, and approved the final manuscript. RA is the guarantor of the manuscript. We wish to acknowledge Mr C P Yadav, Scientist B (Bio-Statistics), National Institute of Malaria Research (NIMR), Delhi for assistance in statistical analysis involving DeNIS database.

Competing Interests All authors have read and understood the The BMJ policy and declare no conflicts of interest.

Provenance and peer review Commissioned; externally peer reviewed.

This article is one of the South Asia series commissioned by The BMJ based on the collaboration of Drugs for Neglected Diseases initiative (DNDi). The BMJ retained full editorial control over external peer review, editing, and publication. Open access fees are funded by the DNDi, Geneva.

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Table 1 Incidence, case fatality rates and ESBL rates of nosocomial sepsis: country specific

<table>
<thead>
<tr>
<th>Countries (no. of studies, no. of isolates)</th>
<th>India (n=68, 18640)</th>
<th>Pakistan (n=15, 3557)</th>
<th>Bangladesh (n=7, 584)</th>
<th>Nepal (n=14, 1331)</th>
<th>Overall SA (n= 105, 24,112)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Incidence of culture positive sepsis, per 1000 live births</strong></td>
<td>17.8 (14.3-21.3); 16</td>
<td>NA</td>
<td>NA</td>
<td>11.6; 1</td>
<td>17.8 (14.3-21.3); 17</td>
</tr>
<tr>
<td><strong>CFR: culture positive sepsis</strong></td>
<td>34.4% (33-35.7); 14</td>
<td>30.9% (25.7-36.2); 2</td>
<td>19.1% (11.7-26.5); 2</td>
<td>64.7% (54.3-75); 1</td>
<td>34.4% (33.1-35.6); 19</td>
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<tr>
<td><strong>CFR: EOS</strong></td>
<td>37.8% (32.7-42.9); 3</td>
<td>39.1% (33.9-44.3); 3</td>
<td>66.7% (49.8-83.5); 1</td>
<td>73.8% (66.6-81); 2</td>
<td>45.4% (42.2-48.6); 9</td>
</tr>
<tr>
<td><strong>CFR: LOS</strong></td>
<td>13.5% (8.3-18.6); 2*</td>
<td>14.9% (11-18.7); 2</td>
<td>22% (7.1-36.8); 1</td>
<td>13.3% (3.9-22.6); 1</td>
<td>26.4% (23.6-29.3); 6</td>
</tr>
<tr>
<td><strong>ESBL rates</strong> (Pooled estimates with 95% CI); N</td>
<td>53.6% (50.7-56.5); 21</td>
<td>NA</td>
<td>NA*</td>
<td>33% (3-63); 1</td>
<td>53.3% (50.4-56.2); 22</td>
</tr>
<tr>
<td><em>Klebsiella spp.</em></td>
<td>42.2% (38.5-46); 16</td>
<td>NA</td>
<td>NA</td>
<td>50% (1-98); 1</td>
<td>42.2% (38.6-46.2); 17</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>65.6 (55.9-75.3); 3</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>65.6 (55.9-75.3); 3</td>
</tr>
<tr>
<td><em>Acinetobacter spp.</em></td>
<td>43.2% (39.9-46.5); 17</td>
<td>61% (49.1-72.8); 1</td>
<td>NA</td>
<td>26% (12.8-39.3); 3</td>
<td>43.3% (40.2-46.5); 21</td>
</tr>
<tr>
<td><strong>MR-Staphylococcus aureus (MRSA)</strong> (Pooled estimates with 95% CI); N</td>
<td>43.2% (39.9-46.5); 17</td>
<td>61% (49.1-72.8); 1</td>
<td>NA</td>
<td>26% (12.8-39.3); 3</td>
<td>43.3% (40.2-46.5); 21</td>
</tr>
</tbody>
</table>

Data represents weighted median (95%CI), N= no. of studies, unless stated otherwise. *Community-based studies (n=3) were not included in the results in this table; *removed outliers for these data-points. SA, South Asia; CFR, case fatality rate; EOS, early onset sepsis; LOS, late onset sepsis; ESBL, extended spectrum beta-lactamase; MR, methicillin resistant; NA, not available.
### Table 2: Pathogen specific CFR and AMR pattern

<table>
<thead>
<tr>
<th>Pathogen (# of isolates)</th>
<th>Ampicillin</th>
<th>Gentamicin</th>
<th>3rd gen. cephalosporins</th>
<th>Meropenem/ Methicillin</th>
<th>MDR</th>
<th>XDR</th>
<th>EOS</th>
<th>LOS</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cefotaxime</td>
<td>Ceftazidime</td>
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<tr>
<td><strong>Klebsiella spp.</strong></td>
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<td>(total n= 4312)</td>
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<td></td>
<td>86.8% (85.8-87.3); 2806</td>
<td>75.3% (74-76.7); 2954</td>
<td>72.5% (71.3-73.7); 4126</td>
<td>74.5% (73-75.9); 2455</td>
<td>10.4% (9.4-11.5); 2540</td>
<td>70.7% (66.1-75.3)</td>
<td>5.2% (2.5-7.8)</td>
<td>69.3% (59.8-78.8)</td>
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<tr>
<td><strong>Escherichia coli</strong></td>
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<td>(n= 2798)</td>
<td>88.2% (87-89.5); 2196</td>
<td>67.9% (66-69.8); 2254</td>
<td>66.9% (65.3-68.6); 2745</td>
<td>69.4% (67.4-71.4); 1773</td>
<td>8.1% (6.8-9.4); 1551</td>
<td>54.0% (48.1-59.9)</td>
<td>2.9% (0.7-5.2)</td>
<td>45.24% (35.5-54.9)</td>
</tr>
<tr>
<td><strong>Acinetobacter spp.</strong></td>
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<tr>
<td>(n=1347)</td>
<td>86.2% (83.8-88.5); 633</td>
<td>68.1% (65.1-71); 792</td>
<td>80.3% (78.2-82.4); 1121</td>
<td>73.6% (70.8-76.3); 718</td>
<td>64.8% (62.2-67.4); 828</td>
<td>78.7% (73.9-83.4)</td>
<td>17.5% (13.1-21.9)</td>
<td>81.4% (75.5-87.4)</td>
</tr>
<tr>
<td><strong>Staph. aureus</strong> (n=2437)</td>
<td>69% (67.3-70.6); 2266</td>
<td>54.5% (52.4-56.6); 1773</td>
<td>51.2% (49-53.3); 1753</td>
<td>NA</td>
<td>46.5% (41.9-51.1); 310</td>
<td>NA</td>
<td>NA</td>
<td>39.2% (28.0-50.4)</td>
</tr>
</tbody>
</table>

Data represents weighted median (95%CI), N= no. of isolates tested, unless stated otherwise. MDR, multidrug resistance; XDR, extreme drug resistance; EOS, Early onset sepsis; LOS, late onset sepsis; NA, Not applicable.
Panel 1: Schematic diagram to describe the evolution and spread of pathogens into multidrug resistant organisms (MDROs), and its exposure leading to sepsis (especially early-onset). The three arrows in red with yellow outline and numbered 1-3 are the points our three-pronged approach highlights: 1 - diagnostic-based antibiotic stewardship program (ASP) to slow MDRO evolution, 2 - decipher transmission dynamics to tackle spread with target of primary prevention and 3 - devise/facilitate newer treatment options through collaborative clinical trials platform. IPC: Infection Prevention and Control; MDRO: Multidrug resistant organisms, NICU: neonatal intensive care unit. NB: The pictures in the diagram have been inserted from internet resources and are indicative only; if accepted, we shall replace them with our own hand-drawn similar sketches.
Panel 2: Insights from DeNIS collaboration study with relevance to contextual issues in neonatal sepsis in South Asia. * ciprofloxacin or cloxacillin or ceftriaxone or amikacin or piperacillin-tazobactam; # vancomycin or meropenem or cefoperazone+sulbactam; NB: neonates analyzed had received at least two days’ therapy of the first-line or second-line options.

<table>
<thead>
<tr>
<th>Items</th>
<th>Overall (n=840)</th>
<th>EOS (n=580)</th>
<th>LOS (n=384)</th>
</tr>
</thead>
<tbody>
<tr>
<td>When first line* alone covered the isolate (concordant)</td>
<td>16/67 (23.9%)</td>
<td>8/49 (16.3%)</td>
<td>8/18 (44.4%)</td>
</tr>
<tr>
<td>When first line did not cover but the second line# covered the isolate (partially discordant)</td>
<td>42/133 (31.6%)</td>
<td>23/65 (35.4%)</td>
<td>19/68 (28.0%)</td>
</tr>
<tr>
<td>When neither the first nor second lines covered the isolate (fully discordant)</td>
<td>90/157 (57.3%)</td>
<td>62/107 (57.9%)</td>
<td>28/50 (56.0%)</td>
</tr>
</tbody>
</table>

Item 3a: Empiric Antibiotics given to all neonates suspected of sepsis (N=1408/13530) correlated with final diagnosis of sepsis

Item 3b: Onset of culture-positive sepsis by age

Item 3c: Case fatality rates among culture positive neonates treated for antibiotic choices (for at least 2 days)
References to main text


Tackling Multidrug Resistance in Neonatal Sepsis: A South Asia Perspective

Supplementary File

Supplementary Panel 1 Systematic review: Search strategy, selection criteria and flow diagram

Search strategy and selection criteria
Systematic search was conducted using PubMed as the primary database with the search terms; (((newborn OR neonate) AND (sepsis OR infection OR antibiotic OR antimicrobial))) in English language between January 1, 2000 to April 2018, last search done on 27th April 2018. The results were filtered for South Asian (SA) Countries namely; (((Afghanistan) OR (Bangladesh) OR (Bhutan) OR (India) OR (Maldives) OR (Nepal) OR (Pakistan) OR (Sri Lanka))). Relevant articles for Nepal and Bangladesh were also searched in the website: https://www.nepjol.info/index.php/JNPS and https://www.banglajol.info/index.php/index. The abstracts and titles were compiled in EndNote (Thomson Reuters) and reviewed to identify relevant studies. Bibliography of full text articles and published systematic reviews were searched to identify additional articles.

Inclusion criteria
● From a country listed in SA region
● Reports data on neonates (age 0-28 days)
● Reports on blood/sterile fluid culture positive sepsis (with or without pneumonia, meningitis or infection at other body sites) with profile of bacterial isolates and (or) antimicrobial resistance (AMR) data
● Reports on the total number of blood cultures obtained and the total number of pathogenic isolates and or AMR data
● Reports should involve recognized standard for the interpretation of antibiotic susceptibility testing like Clinical and Laboratory Standards Institute or British Society for Antimicrobial Chemotherapy or other recognized standard.

Exclusion criteria
● Studies reporting on bacterial isolates retrieved from body surface or other body fluids including urine in neonates without clinical evidence of sepsis or bacteraemia.
● Studies where data specific to neonatal period (0-28 days) could not be extracted
● Studies with less than 15 bacterial isolates
● Studies reporting an outbreak or an epidemic
● Studies whose full text could not be retrieved
● Studies with ambiguous or difficult to extract AMR data

Definitions used:
● Neonates: Age between 0-28 days of life
● Culture positive sepsis: isolation of pathogenic bacteria from blood or other sterile body fluids in a neonate with presumed sepsis with or without features of pneumonia or meningitis.
● Culture negative sepsis: Neonates with clinical features of sepsis but without bacteriological confirmation and receive a course of antibiotics for treatment
● Antimicrobial resistance (AMR): Antimicrobial resistance was defined as resistance of isolated pathogenic bacteria to a particular antibiotic using a standardized antimicrobial susceptibility-testing model such as the agar diffusion test or standardized method for determining the zone of inhibition or minimum inhibitory concentration (MIC) of the isolate; ‘Intermediate’ results if available were clubbed with ‘Resistant’.
● Early and late onset sepsis were defined as per the author’s classification based on either 72 hour cut off or 7 day cut off.
- **Multidrug resistance (MDR)** was defined as per the classification or definition used by the study author.
- **Extremely-drug-resistant (XDR)** was defined resistance to all but one or two antibiotics tested.

**Data extraction**: Data extraction was performed by a single author (SS) using Microsoft Excel 2007 spreadsheet specifically designed for this review that collected information on author, year of study, location, country, study setting, population, incidence of culture positive sepsis, culture positivity rate, number and profile of bacterial pathogens isolated, sepsis related mortality, case fatality rate and antimicrobial resistance pattern. Data extraction was cross-checked for ten percent studies by another author (SC).

**Statistical Analysis**

Reports on incidence, case-fatality rates and bacterial profile of neonatal sepsis were segregated based on whether the study was conducted (or included neonates born) in the community or hospital. When the data included both, the study was analysed under hospital based data. The pooled results were weighted for the number of live births for incidence data and for the number of positive bacterial cultures for bacteriological profile. The case-fatality rates for individual bacteria were weighted for the number of bacterial isolates reported in the study for that pathogen. For antimicrobial resistance pattern, the number of isolates and the percentage of resistance of individual bacterial isolates to various antibiotics were extracted. We calculated the median and 95% CI of AMR weighted by total sample size of blood cultures.

**Results**: The electronic search in PubMed database (latest search on April 27, 2018) identified 2577 articles and an additional 19 articles were identified from reference search of articles identified from PubMed (Figure 1). After excluding 60 duplicate reports, 2536 articles were searched using their title and abstracts. A total of 2370 records were excluded mostly because they included non-bacterial infection or included studies done in older children. One hundred and ninety nine full text articles were reviewed and 94 excluded for various reasons. Finally 105 studies were included. Among these, the Young Infant Clinical Study group 8 provided data for 3 countries, India, Pakistan and Bangladesh on pathogen profile. Five other studies 8,9,10 provided data separately for 2 time periods from a single centre or data for 2 different centres in a single paper and the Neonatal Perinatal Database of India (NNPD) 11 provided data separately for inborn and out-born neonates. No studies for Sri Lanka, Maldives or Afghanistan were found during the search period. Since the data set belonging to a different time period or different patient population was considered unique, a total of 114 individual reports (India-76, Pakistan-16, Nepal-14 and Bangladesh-8) were analysed from 105 studies. The “N” referred to in this article refers to total number of reports from which data was extracted unless specified as number of studies.

**General**: Most studies were from single neonatal units reporting data on neonates with presumed or 9 data from in-born or out-born neonates admitted in the neonatal intensive care unit. The included studies reported on neonates with clinical signs of sepsis with positive bacterial culture or microbiological laboratory based reports of neonates with suspected sepsis. All studies described the source of isolated bacteria, the microbiological techniques and the method of determination of antimicrobial sensitivity or resistance pattern. Among all included studies, pathogenic bacteria were isolated in 24723 cases of sepsis (36.9% [95% CI 36.2 - 37.7]). Gram negative bacteria constituted 62% of all isolates.

**Antimicrobial Resistance**: The overall AMR pattern of important pathogens to various antibiotics in SA region is given in Table 1. The values are weighted percentages of resistance to individual antibiotic weighted by the total number of isolates of a pathogen tested. Supplementary Table 2 lists the details studies included along with the definitions used.

**MDR**: The lack of homogeneity in definition for multidrug resistant (MDR) bacteria in the studies resulted in data that were difficult to compare. Recently in 2012, a group of experts came together to provide consensus and uniformity in defining multidrug resistance (MDR), extreme drug resistance (XDR) and pan-drug resistance (PDR). 8 The group defined MDR as non-susceptibility to at least one agent in three or more antimicrobial categories. XDR was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories and PDR was defined as non-susceptibility to all agents in all antimicrobial categories. The most commonly used MDR definition for gram negative organism is resistance to 3 or more antimicrobial agents. We used XDR definition as mentioned; for DeNIS study 9 we analyzed the XDR rates for pathogens resistant to all antibiotics except colistin (the data used from Joshi et al 10 reported resistant to all antibiotics; however we
considered it into XDR (instead of PDR) assuming since colistin was not tested, these pathogens may have been sensitive to it, given colistin was virtually never used in the pre-2000 era and colisitn resistance has been reported only for last few years).

Limitations: This systematic review has several limitations. It was a limited review of a single database with data extraction done by a single author (SS), but PubMed is the largest database and extraction was randomly cross-checked by the first co-author for 10% studies. Conference proceedings and other grey literature were not searched. Most studies barring a few were hospital based single centre studies managing both inborn and outborn (home delivered or delivered at other health facilities) neonates with or without underlying medical or surgical conditions and of varied gestational age and birth weight. We did not contact the authors of included studies for more information than provided. The review also encompasses data from blood culture based reports providing little clinical information on study subjects. Finally quality assessment of the included studies was not performed.
Records identified through database searching
(n = 2577)

Records after duplicates removed
(n = 2517)

Records identified through other sources
(n = 19)

Records excluded
(n = 2337)

Full-text articles excluded, with reasons
(n = 94)
  - Outbreaks = 12
  - Reviews = 32
  - Children = 17
  - Bacterial isolates not reported individually = 20
  - Infections other than bloodstream = 3
  - Mixed organisms = 1
  - Duplicates = 2
  - Commentary = 1
  - Relevant data not

Studies included in qualitative synthesis
(n = 105)

Flow diagram for the systematic review (based on PRISMA 2009 flow chart - Preferred Reporting Items for Systematic Reviews and Meta-Analyses)
## Supplementary Table 1: Antimicrobial resistance pattern of common pathogens in South Asia

<table>
<thead>
<tr>
<th>Organism</th>
<th>Staph. aureus N= 2437</th>
<th>Klebsiella spp N= 4312</th>
<th>E. coli N= 2798</th>
<th>Acinetobacter spp N= 1347</th>
<th>Pseudomonas spp N= 1445</th>
<th>Enterobacter spp N= 516</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ampicillin</strong></td>
<td>69 (67.3-70.6)</td>
<td>86.8 (85.8-87.3)</td>
<td>88.2 (87-89.5)</td>
<td>86.2 (83.8-88.5)</td>
<td>78.5 (76.6-80.5)</td>
<td>92.3 (90.2-94.4)</td>
</tr>
<tr>
<td></td>
<td>N=2266</td>
<td>N=2806</td>
<td>N=2196</td>
<td>N=633</td>
<td>N=1059</td>
<td>N=497</td>
</tr>
<tr>
<td><strong>Gentamicin</strong></td>
<td>54.5 (52.4-56.6)</td>
<td>75.3 (74-76.7)</td>
<td>67.9 (66-69.8)</td>
<td>68.1 (65.1-71)</td>
<td>60.9 (58.6-63.1)</td>
<td>76.3 (73-79.6)</td>
</tr>
<tr>
<td></td>
<td>N=2954</td>
<td>N=2540</td>
<td>N=2254</td>
<td>N=792</td>
<td>N=1387</td>
<td>N=455</td>
</tr>
<tr>
<td><strong>Amikacin</strong></td>
<td>34 (31.9-36.1)</td>
<td>45.6 (44.3-47)</td>
<td>31.5 (29.7-33.2)</td>
<td>63.5 (61.2-65.7)</td>
<td>36.7 (34.2-39.2)</td>
<td>30 (27-34.4)</td>
</tr>
<tr>
<td></td>
<td>N=1497</td>
<td>N=4128</td>
<td>N=2387</td>
<td>N=1317</td>
<td>N=1147</td>
<td>N=504</td>
</tr>
<tr>
<td><strong>Cefotaxime</strong></td>
<td>51.2 (49-53.3)</td>
<td>72.5 (71.3-73.7)</td>
<td>66.9 (65.3-68.6)</td>
<td>80.3 (78.2-82.4)</td>
<td>58.8 (56.3-61.2)</td>
<td>75.5 (72-79)</td>
</tr>
<tr>
<td></td>
<td>N=1753</td>
<td>N=4126</td>
<td>N=2745</td>
<td>N=1121</td>
<td>N=1154</td>
<td>N=494</td>
</tr>
<tr>
<td><strong>Ciprofloxacin</strong></td>
<td>42.4 (40.6-44.3)</td>
<td>53.8 (52.4-55.2)</td>
<td>40.5 (38.8-42.3)</td>
<td>70.3 (68.1-72.5)</td>
<td>33.5 (31.4-35.6)</td>
<td>38.7 (35.4-42)</td>
</tr>
<tr>
<td></td>
<td>N=2196</td>
<td>N=4054</td>
<td>N=2720</td>
<td>N=1332</td>
<td>N=1435</td>
<td>N=504</td>
</tr>
<tr>
<td><strong>Meropenem</strong></td>
<td>10.4 (9.4-11.5)</td>
<td>8.1 (6.8-9.4)</td>
<td>64.8 (62.2-67.4)</td>
<td>17.6 (15.3-20)</td>
<td>9.5 (4.9-14.2)</td>
<td>-</td>
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<tr>
<td></td>
<td>N=2540</td>
<td>N=1551</td>
<td>N=828</td>
<td>N=560</td>
<td>N=127</td>
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<tr>
<td><strong>Ceftazidime</strong></td>
<td>74.5 (73-75.9)</td>
<td>69.4 (67.4-71.4)</td>
<td>73.6 (70.8-76.3)</td>
<td>49.5 (47.1-51.8)</td>
<td>66.4 (62.4-70.4)</td>
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<tr>
<td></td>
<td>N=2455</td>
<td>N=1773</td>
<td>N=718</td>
<td>N=1170</td>
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<td></td>
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<tr>
<td><strong>Piperacillin-taobactam</strong></td>
<td>25 (23-26.8)</td>
<td>57.4 (53.5-61.2)</td>
<td>64.6 (62.1-67.2)</td>
<td>28 (24.7-31.2)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=1608</td>
<td>N=400</td>
<td>N=935</td>
<td>N=468</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Polymyxin</strong></td>
<td>6.6 (4.4-9)</td>
<td>30.6 (14.7-46.6)</td>
<td>2.9 (0-5)</td>
<td>10.1 (6.1-14.1)</td>
<td>33.5 (4.9-62.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=293</td>
<td>N=28</td>
<td>N=200</td>
<td>N=183</td>
<td>N=9</td>
<td></td>
</tr>
<tr>
<td><strong>Tigecycline</strong></td>
<td>6 (3-8.6)</td>
<td>4.3 (2-7)</td>
<td>16.8 (12.9-20.8)</td>
<td>67.4 (60-74.9)</td>
<td>2 (0-5.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=307</td>
<td>N=192</td>
<td>N=317</td>
<td>N=72</td>
<td>N=57</td>
<td></td>
</tr>
<tr>
<td><strong>Cefipime</strong></td>
<td>51.2 (44-58.3)</td>
<td>44.3 (36.9-51.8)</td>
<td>71.1 (64.9-77.3)</td>
<td>27.7 (24-31.4)</td>
<td>45.4 (29.6-61.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=143</td>
<td>N=88</td>
<td>N=172</td>
<td>N=180</td>
<td>N=11</td>
<td></td>
</tr>
<tr>
<td><strong>Cloxacillin</strong></td>
<td>61.2 (58.5-63.9)</td>
<td>Not Applicable</td>
<td>Not Applicable</td>
<td>Not Applicable</td>
<td>Not Applicable</td>
<td>Not Applicable</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>Resistance (%)</td>
<td>95% CI</td>
<td>N</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>--------------</td>
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<td>---------------</td>
<td>-----</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methicillin</td>
<td>46.5</td>
<td>(41.9-51.1)</td>
<td>310</td>
<td></td>
<td></td>
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<tr>
<td>Linezolid</td>
<td>2.8</td>
<td>(1.4-4.7)</td>
<td>281</td>
<td></td>
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</tr>
<tr>
<td>Vancomycin</td>
<td>19.1</td>
<td>(17.4-20.8)</td>
<td>1575</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data represents weighted median (95%CI). N= no. of isolates tested, unless stated otherwise; colour code: less than 50% resistance is shaded green, greater than or equal to 50% is shaded orange.
<table>
<thead>
<tr>
<th>Author, country</th>
<th>Definition of MDR</th>
<th>Proportions of MDR organisms</th>
</tr>
</thead>
</table>
| Viswanathan et al (2012, India) | resistance to all 3 groups of antibiotics studied namely ampicillin, gentamicin and cefotaxime/ceftazidime | · Klebsiella spp. (58/60, 96%)  
· E. coli (18/25, 72%)  
· Acinetobacter spp. (13/15, 87%) |
| Viswanathan et al (2014), India | resistance to any 3 classes: 3rd generation cephalosporins, aminoglycosides and fluoroquinolones | · Acinetobacter spp. (15/30, 50%)  
· Carbapenem Resistance (9/30, 30%)  
· Pan-resistance (5/30, 16.6%) |
| Roy et al (2015), India | resistance to 2 or more classes of drugs | · E. coli (52/67, 78%); most common (24%, 16/67)  
resistance pattern: 'ciprofloxacin-ceftaxime-amikacin-trimethoprim/sulfamethoxazole' |
| DeNIS collaboration (2016), India | any 2 of 5 classes: Extended spectrum cephalosporins, carbapenem, aminoglycoside, fluoroquinolone and piperacillin-tazobactam | · Acinetobacter spp (181/222, 82%)  
· Klebsiella spp (91/169, 54%)  
· Escherichia coli (52/137, 38%)  
· Pseudomonas spp. (13/68, 19%)  
· Enterobacter spp (22/44, 50%) |
| Dhaneria et al (2018), India | resistance to at least 3 antibiotics | · Klebsiella spp (9/11, 86%)  
· Pseudomonas spp. (5/6, 82%)  
· E. coli (4/5, 80%)  
· Proteus spp (3/4, 75%) |
| Khanal et al (2015), Nepal | resistance to 2 or more drugs | · Gram-positive (40/61, 65%)  
· Gram-negative (8/8, 100%)  
· Overall 48/69, 69.5% |
| Joshi et al (2000), India | resistance to all antibiotics tested | · Klebsiella spp. (7/70, 10%)  
· E. coli (2/36, 6.2%)  
· Acinetobacter spp (4/18, 22.2%)  
· Pseudomonas spp. (19/88, 22%) |
| Ansari et al (2015), Nepal | most antibiotics | · Klebsiella spp (3/9, 33%)  
· Enterobacter spp (6/6, 100%)  
· E. coli (2/4, 50%)  
· Acinetobacter spp. (3/4, 75%) |
| Srivastava et al (2014), India | more than 3 groups of antibiotics | · Gram negative bacilli (27/108, 25%) |
| Haque et al (2014), Bangladesh | resistance to 3 or more OR combined resistance against ceftazidime and ceftriaxone | · K. pneumoniae (67/79, 85%) |
References to Appendix and of articles included in systematic review


