Neonatal Risk Factors for Colonization with Extended-Spectrum Beta-Lactamase-Producing Bacteria in the Neonatal Intensive Care Unit

Viktoria Leikin-Zach MD1*, Elon Shany MD1,2, Maayan Yitshak-Sade PhD3,6, Ron Eshel B Med Sc3, Tali Shafat MD3, Avraham Borer MD1,4 and Rimma Melamed MD1,5

1Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel
2Department of Neonatology, 3Clinical Research Center, 4Infection Control and Hospital Epidemiology Unit and 5Pediatric Infectious Diseases Unit, Soroka Medical Center, Beer Sheva, Israel
6Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts, USA

ABSTRACT: Background: Extended-spectrum beta-lactamase (ESBL) production is the most common antimicrobial resistance mechanism in the neonatal intensive care unit (NICU), with colonization and blood stream infections being a major threat to this population. Since 2013, all NICU admissions at our facility were screened twice weekly for ESBL colonization.

Objectives: To determine independent risk factors for colonization of infants with ESBL-producing bacteria in the NICU.

Methods: A retrospective case study of ESBL-colonized infants vs. controls (matched by date of birth and gestational age) was conducted in the NICU of Soroka University Medical Center, Israel, between 2013 and 2014. Epidemiological, laboratory, and clinical data were extracted from medical files. Univariable and multivariable analyses were used to assess associations between ESBL colonization and possible clinical risk factors.

Results: Of 639 admissions during the study period, 87 were found to be ESBL-colonized (case infants) and were matched to 87 controls. Five case infants became infected (5.7%) with ESBL strains. Klebsiella pneumoniae was the most common isolated bacteria. The mean time from admission to colonization was 15 days. Univariable analysis showed an association of male gender and highest Apgar score at 1 and 5 minutes with ESBL colonization (P < 0.05). Multivariable analysis yielded only a possible association of higher Apgar score at 1 and 5 minutes (hazard ratio [HR] 1.515, 95% confidence interval [95% CI] 0.993–2.314; HR 1.603, 95% CI 0.958–2.682, respectively) with ESBL colonization.

Conclusions: Future studies should focus on maternal colonization and possible strategies for preventing vertical transmission of ESBL strains to high-risk neonates.

KEY WORDS: neonatal intensive care unit (NICU), antibiotics, extended spectrum beta-lactamase (ESBL), antimicrobial resistance, screening

Resistance to beta-lactam antibiotics occurs primarily through the production of beta-lactamases enzymes that split the amide bond of the beta-lactam ring. Beta-lactamases most likely co-evolved with bacteria as mechanisms of resistance against natural antibiotics and the selective pressure exerted by the widespread use of antimicrobial therapy in the modern era may have accelerated their spread [1].

Extended-spectrum beta-lactamases (ESBL) are enzymes that can hydrolyze the beta-lactam ring of extended-spectrum (third generation) cephalosporins and monobactams [2], resulting in carbapenems as the main remaining treatment option [3]. These enzymes are encoded either by chromosomal point mutations or by transferable genes located on plasmids and transposons [4,5].

The first ESBL was identified during a hospital outbreak of Klebsiella pneumoniae (K. pneumoniae) in Germany in 1982 [6]. Since then, more than 200 ESBL variants have been identified, some of which spread rapidly worldwide. Many ESBL variants initially identified in K. pneumoniae were subsequently also found in Escherichia coli (E. coli).

Outbreaks in neonatal intensive care units (NICU) of ESBL-producing organisms are an emerging threat [7-9]. ESBL production is described as the most common antimicrobial resistance mechanism in the NICU [10], and higher mortality and morbidity rates are reported in infants affected by ESBL-producing strains [11,12].

A summary of systematic reviews by the World Health Organization (WHO) states that patients with resistant K. pneumonia infections carry a risk of worse clinical outcomes and consume more healthcare resources than patients infected by susceptible strains [3]. In their review on community-acquired neonatal and infant sepsis in developing countries, Downie and co-authors [13] concluded that due to resistant strains, a significant proportion of blood steam infections were not treatable by a recommended first-line regimen nor by second line alternatives (cephalosporins).
Previous studies focused mainly on factors associated with the risk of infection by ESBL-producing strains, but they lacked information on factors associated with the risk of ESBL colonization [9,14-16].

We hypothesized that some of the epidemiological and clinical features of NICU patients and their mothers were associated with colonization of ESBL-producing bacteria.

The objective of our study was to determine independent risk factors for colonization of infants with ESBL-producing bacteria in the NICU.

**PATIENTS AND METHODS**

The study was approved by the local institutional review board at Soroka University Medical Center (0439-15 SOR).

**STUDY DESIGN**

This was a retrospective, matched, case-control study.

**STUDY POPULATION**

Included in the study were neonates hospitalized in the NICU of Soroka University Medical Center between 1 July 2013 and 31 December 2014.

Soroka is the only medical center in the Negev area of southern Israel and provides medical services to a population in excess of 1 million inhabitants, with a yearly delivery rate of 13,500–17,000 neonates.

Since 2013, every new admission to the NICU has been screened for ESBL-producing bacteria using a rectal swab starting on the day of admission and then every 3 days until discharge. Colonization was defined as at least one new documented positive rectal swab for ESBL-producing bacteria during the hospitalization period in the NICU. For each case (colonized infant), one control infant whose screens were all negative was matched by date of admission (± 3 weeks) and gestational age (± 3 weeks).

**DATA COLLECTION**

The epidemiological, laboratory, and clinical data of infants and their mothers were collected retrospectively from their computerized medical records. The information included date of birth, time to discharge, and first positive screening culture (for cases). Additional data included birth weight (dichotomized to small for gestational age), gestational age, gender, ethnicity (Jewish/Bedouin), Apgar scores at 1 and 5 minutes (dichotomized to less than 5 at 1 minute and less than 8 at 5 minutes, the cut off was chosen as the upper quartile vs. the three lower ones), invasive (with endotracheal tube) and non-invasive (continuous positive airway pressure of high-flow nasal cannula) respiratory support, duration of oxygen supplementation, parenteral nutrition duration, umbilical line catheterization duration (venous, arterial), peripherally inserted central line duration usage, and duration of antimicrobial treatment. Obstetric data included mode of delivery (vaginal vs. cesarean section), preterm premature rupture of the membranes (PPROM), maternal corticosteroid treatment, and antimicrobial treatments prior to delivery.

Follow-up time was defined for each case as the number of days from NICU admission to the first positive screening culture and the total number of days of each condition during this time. To avoid potential bias due to a longer follow-up period for controls, follow-up time was matched to the case time to colonization.

**STATISTICAL ANALYSIS**

Statistical analyses were performed using IBM Statistical Package for the Social Sciences statistics software, version 21 (SPSS, IBM Corp, Armonk, NY, USA) and SAS 9.4 software (SAS Institute Inc., Cary, NC, USA).

Results are presented as mean ± standard deviation for normally distributed continuous variables, as median and inter quartile range (IQR) for continuous variables that are not normally distributed, and as percentages for categorical data.

Univariable analysis by Cox regression was used to assess associations between ESBL colonization and possible clinical risk factors. Robust variance was used to account for the matched pairs. Variables with P ≤ 0.1 in the univariable analysis were further evaluated in a multivariable model.

Two-sided tests were used for all analyses. P < 0.05 was considered statistically significant. Results are presented as hazard ratios (HR) and 95% confidence intervals (95%CI).

**RESULTS**

A total of 639 infants were admitted to the NICU during the study period. Eighty-seven infants who were found to be colonized with ESBL strains during this period were matched with 87 controls.

Demographic and baseline clinical characteristics of infants colonized with ESBL-producing bacteria and their controls are shown in Table 1. Gestational age of the whole cohort ranged between 24 and 43 weeks. Cases were more likely to be male (64% vs. 47%, P = 0.022). Five infants (5.7%) of the colonized group had a bloodstream infection with resistant strains. The median duration of NICU stay until ESBL colonization was 15 days (IQR 7–26). Overall, colonized infants had a longer hospitalization in the NICU (37 vs. 16 days, P < 0.001).

Of the 87 cases, 57 were colonized with *K. pneumonia* (65.5%), 26 with *E. coli* (29.9%), 2 with *Kluyvera Georgiana* (2.3%), 1 with *Klebsiella oxytoca* (1.1%), and 1 with *Enterobacter cloacae* (1.1%).

In the univariable Cox regression models with robust variance (Table 2), three variables were found as possible risk factors for colonization of ESB-producing bacteria: male gender (HR 1.53, 95% CI 0.99–2.36), higher 1 minute Apgar score (HR 1.53, 95% CI 0.99–2.36), and duration of NICU stay until ESBL colonization (HR 1.53, 95% CI 0.99–2.36).
**Table 1.** Demographic and clinical characteristics of cases vs. control

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (n=87)</th>
<th>Controls (n=87)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age, weeks</td>
<td>31.2 ± 4.4</td>
<td>31.6 ± 4.2</td>
<td>0.511</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>56 (64.4)</td>
<td>41 (47.1)</td>
<td>0.022</td>
</tr>
<tr>
<td>Vaginal delivery, n (%)</td>
<td>37 (44.8)</td>
<td>37 (42.5)</td>
<td>0.36</td>
</tr>
<tr>
<td>Small for gestational age, n (%)</td>
<td>23 (26.4)</td>
<td>21 (24.1)</td>
<td>0.154</td>
</tr>
<tr>
<td>Bedouin ethnicity, n (%)</td>
<td>51 (58.6)</td>
<td>77 (80.1)</td>
<td>0.642</td>
</tr>
<tr>
<td>PPROM, n (%)</td>
<td>16 (18.4)</td>
<td>11 (12.6)</td>
<td>0.295</td>
</tr>
<tr>
<td>1 minute Apgar score, median (IQR)</td>
<td>6 (5–9)</td>
<td>6 (4–9)</td>
<td>0.754</td>
</tr>
<tr>
<td>5 minute Apgar score, median (IQR)</td>
<td>9 (8–10)</td>
<td>9 (8–10)</td>
<td>0.439</td>
</tr>
<tr>
<td>Duration of hospitalization in the NICU, median (IQR)</td>
<td>37 (12–58)</td>
<td>16 (6–36)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Data analyzed with Student’s t-test, chi-square and Mann-Whitney U test as appropriate

PPROM = preterm premature rupture of the membranes, NICU = neonatal intensive care unit, IQR = interquartile range

**Table 2.** Risk factors for colonization with ESBL-producing bacteria

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>HR</th>
<th>95%CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive respiratory support</td>
<td>1.04</td>
<td>0.68–1.58</td>
<td>0.866</td>
</tr>
<tr>
<td>Non-invasive respiratory support</td>
<td>0.8</td>
<td>0.49–1.33</td>
<td>0.398</td>
</tr>
<tr>
<td>Maternal antibiotic treatment</td>
<td>0.92</td>
<td>0.60–1.38</td>
<td>0.675</td>
</tr>
<tr>
<td>Maternal prenatal steroid treatment</td>
<td>0.72</td>
<td>0.46–1.12</td>
<td>0.141</td>
</tr>
<tr>
<td>Male gender</td>
<td>1.53</td>
<td>0.99–2.36</td>
<td>0.056</td>
</tr>
<tr>
<td>Bedouin ethnicity</td>
<td>1.21</td>
<td>0.79–1.84</td>
<td>0.383</td>
</tr>
<tr>
<td>Total days of antibiotic treatment</td>
<td>0.65</td>
<td>0.35–1.19</td>
<td>0.161</td>
</tr>
<tr>
<td>Duration of oxygen treatment</td>
<td>0.59</td>
<td>0.33–1.07</td>
<td>0.082</td>
</tr>
<tr>
<td>Days of umbilical vein catheter use</td>
<td>0.78</td>
<td>0.47–1.30</td>
<td>0.345</td>
</tr>
<tr>
<td>Days of umbilical artery catheter use</td>
<td>0.63</td>
<td>0.39–1.02</td>
<td>0.062</td>
</tr>
<tr>
<td>Days of peripherally inserted central line use</td>
<td>0.64</td>
<td>0.39–1.05</td>
<td>0.078</td>
</tr>
<tr>
<td>Apgar score &gt; 5 at 1 minute</td>
<td>1.653</td>
<td>1.10–2.49</td>
<td>0.0168</td>
</tr>
<tr>
<td>Apgar score &gt; 8 at 5 minutes</td>
<td>1.963</td>
<td>1.12–3.11</td>
<td>0.017</td>
</tr>
<tr>
<td>PPROM</td>
<td>1.35</td>
<td>0.82–2.21</td>
<td>0.233</td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>1.04</td>
<td>0.70–1.55</td>
<td>0.834</td>
</tr>
<tr>
<td>Small for gestational age</td>
<td>1.01</td>
<td>0.68–1.53</td>
<td>0.943</td>
</tr>
</tbody>
</table>

Univariable hazard Cox model with robust variance

ESBL = extended-spectrum beta-lactamase, PPROM = preterm premature rupture of the membranes, NICU = neonatal intensive care unit, IQR = interquartile range

1.653, 95%CI: 1.095–2.494), and higher 5 minute Apgar score (HR 1.863, 95%CI 1.117–3.107).

Due to a relatively high correlation between Apgar score at 1 and 5 minutes (Spearman coefficient 0.652, P < 0.0001), two multivariable models were created [Tables 3A and 3B]. In these models, Apgar scores at 1 and 5 minutes tended to significance (HR 1.515, 95%CI 0.993–2.314; HR 1.603, 95%CI 0.958–2.682, respectively). None of the other variables were found to be significantly associated with higher risk of colonization with ESBL-producing bacteria.

**Table 3A.** Risk factors for ESBL-producing bacteria colonization (Apgar score at 1 minute)

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>HR</th>
<th>95%CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>1.415</td>
<td>0.887–2.256</td>
<td>0.1453</td>
</tr>
<tr>
<td>Apgar score &gt; 5 at 1 minute</td>
<td>1.515</td>
<td>0.993–2.314</td>
<td>0.0542</td>
</tr>
<tr>
<td>Duration of oxygen treatment</td>
<td>0.573</td>
<td>0.305–1.075</td>
<td>0.0628</td>
</tr>
<tr>
<td>Days of central line use</td>
<td>0.830</td>
<td>0.492–1.400</td>
<td>0.4851</td>
</tr>
</tbody>
</table>

Multivariable hazard Cox model with robust variance

ESBL = extended-spectrum beta-lactamase, HR = hazard ratio, 95%CI = 95% confidence interval

**Table 3B.** Risk factors for ESBL-producing bacteria colonization (Apgar at 5 minutes)

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>HR</th>
<th>95%CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>1.426</td>
<td>0.878–2.317</td>
<td>0.1515</td>
</tr>
<tr>
<td>Apgar score &gt; 8 at 5 minutes</td>
<td>1.603</td>
<td>0.958–2.682</td>
<td>0.0724</td>
</tr>
<tr>
<td>Duration of oxygen treatment</td>
<td>0.642</td>
<td>0.335–1.233</td>
<td>0.1828</td>
</tr>
<tr>
<td>Days of central line use</td>
<td>0.783</td>
<td>0.475–1.293</td>
<td>0.3394</td>
</tr>
</tbody>
</table>

Multivariable hazard Cox model with robust variance

ESBL = extended-spectrum beta-lactamase, HR = hazard ratio, 95%CI = 95% confidence interval

**DISCUSSION**

In this retrospective case control study of risk factors for colonization of newborn infants with ESBL-producing agents, *K. pneumoniae* was the most common organism isolated and median time from admission to colonization was 15 days.

Colonization with ESBL-producing strains was associated with male gender and higher Apgar score at 1 and 5 minutes. In multivariable analysis, Apgar scores at 1 and 5 minutes trended as an independent risk factor of ESBL colonization.

We found a colonization rate for *K. pneumoniae* of 8.89% in our study population. Benenson and co-authors [17] found a colonization rate of 11% at a NICU in Jerusalem, Israel, and Somily and colleagues [18] reported a prevalence of 6.8% in Riyadh, Saudi Arabia. Stapleton and co-authors [12] conducted a meta-analysis of ESBL outbreaks. They found *K. pneumoniae* to be the most frequently implicated pathogen, similar to our study. The most commonly identified source of colonization of infants in this meta-analysis was horizontal dissemination (15% of sources). Although during our study period mothers were not screened for ESBL strains, we can estimate a 6.5% rate of vertical transmission as five case infants had a positive ESBL screen on the day of admission. This finding prompted us recently to screen mothers as well as their infants for ESBL-producing bacteria.

Gender difference related to ESBL-producing bacteria was previously reported in a study from Brazil [19] where 55% of blood stream infections occurred in males, a finding that is in line with our reported prevalence of 65% male colonization;
however, in other studies [9, 20] neither gender predominance was found or female predominance was reported [14].

In previous studies, in contrast to ours, use of antibiotics was the most frequently identified independent risk factor for neonatal ESBL colonization [14, 15, 20, 21]. This difference could be due to the various settings of those studies, as all of them were reports of outbreaks that had different dynamics than our NICU, where several ESBL strains coexist with a fraction of probable vertical transmission and a lower proportion of colonized infants. It should also be noted that not only was the cumulative time of antibiotic administration not found to be a risk factor in our cohort, but also there was no difference between the proportion of infants treated with antibiotics in our study in either group. However, as a large proportion was treated with antibiotics (80% in both groups), it is unlikely that antibiotic treatment would play a role as a risk factor in this cohort.

In other studies, in contrast to ours, low birth weight [11] and use of central venous catheters [14] were found to be independent risk factors for colonization in the NICU. These differences can be attributed to the nature of these studies, which described outbreaks rather than the steady state condition of our NICU. Thus, since we practiced routine ESBL colonization monitoring and cohorting of affected cases, we managed to prevent horizontal transmission, which is related to the described risk factors.

Other independent risk factors, such as length of stay in the NICU [22, 23] and younger gestational age [14, 15, 21], could not be analyzed in our study as controls were matched to gestational age and time of first positive ESBL screen.

The finding that higher Apgar scores may be related to higher colonization rates can be linked to the finding that infants with higher Apgar scores were more likely to be delivered vaginally, Apgar 8 (IQR 5–9) vs. 5 (IQR 4–8) (P < 0.001), or being of older gestational age at delivery (P = 0.048) and thus were more likely to be in close contact to their mothers, either immediately after birth or by early Kangaroo mother care. This association was also found in a study by Shany and co-authors [23] in their univariable analysis, but this finding seems weak and should be further explored.

Some of the differences between the studies may be related to the different approaches used regarding the follow-up times of cases and controls. Thus, while we used the time to first positive screen test as a follow-up time for each case and its matched control, Pessoa-Silva et al. [14] used the median time to colonization as a follow-up time for controls and Vijayakanthi and colleagues [22] determined a follow-up time of cases and controls. Thus, while we used the time to first positive ESBL screen, the cumulative time of antibiotic administration not found to be a risk factor in our cohort, but also there was no difference between the proportion of infants treated with antibiotics in our study in either group. However, as a large proportion was treated with antibiotics (80% in both groups), it is unlikely that antibiotic treatment would play a role as a risk factor in this cohort.

In other studies, in contrast to ours, low birth weight [11] and use of central venous catheters [14] were found to be independent risk factors for colonization in the NICU. These differences can be attributed to the nature of these studies, which described outbreaks rather than the steady state condition of our NICU. Thus, since we practiced routine ESBL colonization monitoring and cohorting of affected cases, we managed to prevent horizontal transmission, which is related to the described risk factors.

Other independent risk factors, such as length of stay in the NICU [22, 23] and younger gestational age [14, 15, 21], could not be analyzed in our study as controls were matched to gestational age and time of first positive ESBL screen.

The finding that higher Apgar scores may be related to higher colonization rates can be linked to the finding that infants with higher Apgar scores were more likely to be delivered vaginally, Apgar 8 (IQR 5–9) vs. 5 (IQR 4–8) (P < 0.001), or being of older gestational age at delivery (P = 0.048) and thus were more likely to be in close contact to their mothers, either immediately after birth or by early Kangaroo mother care. This association was also found in a study by Shany and co-authors [23] in their univariable analysis, but this finding seems weak and should be further explored.

Some of the differences between the studies may be related to the different approaches used regarding the follow-up times of cases and controls. Thus, while we used the time to first positive screen test as a follow-up time for each case and its matched control, Pessoa-Silva et al. [14] used the median time to colonization as a follow-up time for controls and Vijayakanthi and colleagues [22] determined a follow-up time of cases and controls until discharge. We therefore did not include the length of hospitalization in the analysis of risk factors, as all parameters that we collected were related to the time until the first positive colonization screen.

The main limitation of this study was the lack of information related to maternal colonization, which was only recently initiated in our service and may be a major factor in the colonization risk of infants in a NICU such as ours where ESBL colonization is not due to an outbreak. Other limitations consist of the retrospective nature of the study and the relatively small number of participants.

The strength of this study relates to the meticulous prospective collection of specimens from neonates in the NICU, which was performed under a strict built-in protocol and included all infants who were admitted to the NICU irrespective of their diagnosis and gestational age.

CONCLUSIONS

The current study found Apgar scores to be the only possible risk factors for ESBL colonization related to delivery and postnatal NICU care. We think that this is rather a surrogate marker for a combination of several other factors that we could not isolate. We believe that future studies in non-outbreak conditions should focus on maternal colonization and possible strategies of preventing vertical transmission of ESBL strains to high-risk neonates.

Correspondence
Dr. E. Shany
Dept. of Neonatology, Soroka University Medical Center, Beer Sheva 84101, Israel
Phone: (972-8) 640-0549
Fax: (972-8) 640-0545
email: eshany@bgu.ac.il

References


**Capsule**

**Estrogen accentuates autoimmunity**

More than any other risk factor, being female confers the greatest risk of developing an autoimmune disorder. Mohammad and co-authors identified a role of estrogen in the development of autoimmune T cell responses. Deletion of estrogen receptor α (ERα) in T cells reduced disease burden in a mouse model of colitis. ERα-expressing T cells were more activated after stimulation and produced more proinflammatory cytokines than T cells lacking this receptor. Conversely, ERα-deficient T cells were more likely to differentiate into regulatory T cells, which suppress the development of autoimmune disorders.

*Sci Signal* 2018; 11: eaap415

Eitan Israeli

**Capsule**

**Resistance of HIV-infected macrophages to CD8+ T lymphocyte-mediated killing drives activation of the immune system**

CD4+ T lymphocytes are the principal target of the human immunodeficiency virus (HIV), but infected macrophages also contribute to viral pathogenesis. The killing of infected cells by CD8+ cytotoxic T lymphocytes (CTLs) leads to control of viral replication. Clayton and colleagues found that the killing of macrophages by CTLs was impaired relative to the killing of CD4+ T cells by CTLs, and this resulted in inefficient suppression of HIV. The killing of macrophages depended on caspase-3 and granzyme B, whereas the rapid killing of CD4+ T cells was caspase independent and did not require granzyme B. Moreover, the impaired killing of macrophages was associated with prolonged effector cell-target cell contact time and higher expression of interferon-γ by CTLs, which induced macrophage production of pro-inflammatory chemokines that recruited monocytes and T cells. Similar results were obtained when macrophages presented other viral antigens, suggestive of a general mechanism for macrophage persistence as antigen-presenting cells that enhance inflammation and adaptive immunity. Inefficient killing of macrophages by CTLs might contribute to chronic inflammation, a hallmark of chronic disease caused by HIV.

*Nature Immunol* 2018; 19: 475

Eitan Israeli

“**It is better to know some of the questions than all of the answers**”

James Grover Thurber, (1894–1961), American cartoonist, author, humorist, journalist, playwright, and celebrated wit

“**It is health that is real wealth and not pieces of gold and silver**”

Mohandas Karamchand Gandhi, (1869–1948), Leader of the Indian independence movement against British rule. Employing non-violent civil disobedience, he led India to independence and inspired movements for civil rights and freedom across the world.